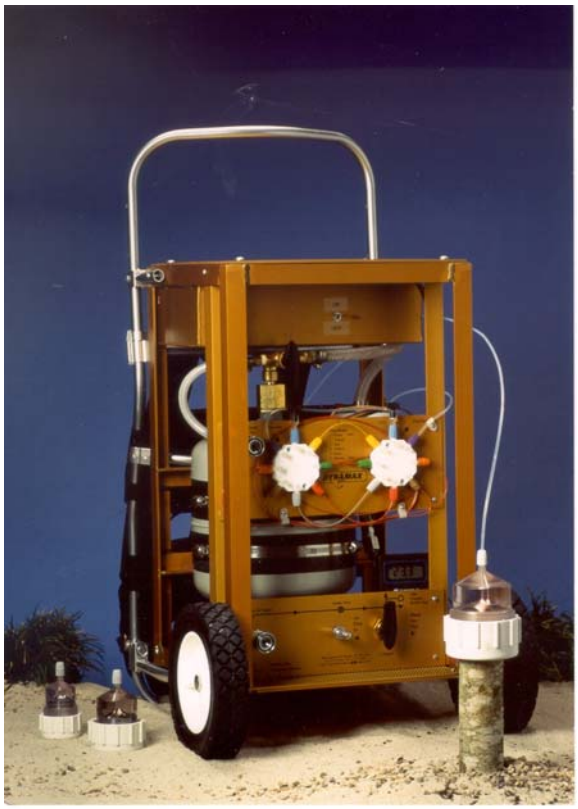
High Pressure Flow Meter
Installation and Operations Manual



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1. Introduction

1.1 High Pressure Flow Meter (HPFM) Form & Function

Thank you for purchasing a Dynamax High Pressure Flow Meter (HPFM). The HPFM is shown schematically in Figure 1. It is an apparatus designed to perfuse water into an object while rapidly changing the delivery pressure and simultaneously measuring flow. The slope of flow plotted versus pressure equals the hydraulic conductance of the object. The object can be an excised shoot or excised root system or capillary tube (for test purposes). The HPFM is used to measure quasi-steady state conductance, i.e., under conditions where flow and applied pressure are approximately constant with time.

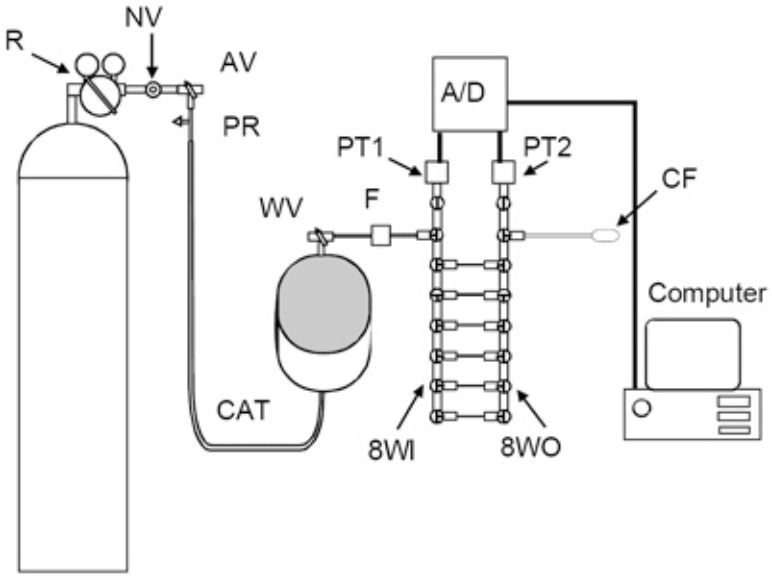


Figure 1
Schematic of High Pressure Flow Meter (HPFM)

The rapid change in water pressure is achieved using a pressure regulator (R), a needle valve (NV), and a captive air tank (CAT). A pressure regulator (R) delivers compressed air from the compressed air tank (AT). The pressure regulator keeps the compressed air at a steady pressure of 4 to 5 MPa (580-725 PSI) through a needle valve (NV).

The NV is connected to the air-chamber (A) of a captive air tank (CAT). A rubber diaphragm separates the air from water (W) in the CAT. The NV is adjusted to permit a rate of airflow to pressurize the air volume at a rate of 3 to 7 kPa s^{-1} (approx. 1-PSI s^{-1}). The volumes of air and water are approximately equal in the CAT. The CAT can be pressurized or depressurized by turning the 3-way ball valve (AV).

A pressure release valve (PR) prevents accidental over-pressurization. The pressure release valve is set to vent air when the pressure exceeds about 0.6 MPa (87 PSI). Since the pressure in (A) never exceeds 0.6 MPa, and the air supply is 4.2 MPa (609 PSI), the pressure drop across the NV is approximately constant. This allows the rate of pressurization in the water to be approximately linear with time.

Pressurized water flows from the CAT to the 8-Way Inlet manifold (8WI) through a 9 mm Inside Diameter (ID) nylon-reinforced Tygon tubing. At a distance of 0.3 m from the CAT the water passes through a 0.1 μm water filter, then the diameter of the tubing is reduced to 3 mm Outside Diameter (OD) (1.5mm ID) plastic tube; the plastic tubing is connected to the input side of the 8WI. The 8WI is shown schematically in Figure 1. The 8WI is an Omnifit, 8-Way, HPLC valve of octagonal geometry with 8-tubes emerging from a common point in the center and each tube terminated by a valve.

On the inlet side, the 3 mm OD (1.5 mm ID) tube from the CAT is connected to one of the 8 valves (purple connector on the HPFM). A pressure transducer (PT1) is connected to another valve (blue connector on the HPFM). On the outlet side another pressure transducer (PT2) is connected to 8WI (blue connector) as well as the 3 mm OD (1.5mm ID) outlet tube (purple connector). This leaves 6 pairs of valves available between each 8WI and 8-Way Outlet (8WO) for connection of narrow-bore capillary tubes (CT).

The capillary tubes are 1.5 mm OD HPLC tubing 0.16 to 1.5 m in length. The HPLC CTs have internal diameters from 0.12 to 0.5 mm. During a measurement, one CT is selected by opening the inlet and outlet valves of the corresponding manifold (8WI and 8WO). The corresponding CT valves for the unused capillary tubes must be closed. Water flow across the selected CT causes a differential pressure (dP) drop ($dP = P_1 - P_2$) measured with PT1 and PT2, respectively.

Calibration curves were established relating flow, F, to dP values in the range of 0 to 100 kPa (0-15 PSI). HPLC capillary tubes up to 0.25 mm ID have linear calibration curves. HPLC capillary tubes 0.3 mm ID or larger are nonlinear because of the transition from laminar to turbulent flow.

The pressure measurements are made with model PX26-100GV solid-state strain-gauge transducers. The output of the pressure transducers is logged using a custom-designed, dual channel A/D circuit with 12 bits plus sign accuracy. The HPFM is supplied with Windows software for controlling the A/D circuits, logging data, and for preliminary data analysis.

The Flow Outlet side of the 8-Way manifold is connected by a 6-foot (1.8 m) length of water-filled 1.5 mm ID Teflon FEP “Clear” tubing to a compression fitting (CF). This FEP tubing permits connection to the larger size plant or root base with 19-50 mm fittings (HPCC19-35 and HPCC34-50). Additional (1 mm ID) HPLC “Natural” (light tan) tubing is supplied for use on smaller sized roots and shoots with 1-20 mm fittings (HPCC 1-4, HPCC 4-10 and HPCC 10-20). We also supply 0.127 mm ID ‘RED’ tubing, which is used to assist air bubble removal in the system AND to use for test measurement. This red tube has the conductance of a small seedling with about 100 to 200 cm^2 of leaf area. A range of compression fittings is supplied to permit connection to stems ranging from 1 to 50 mm OD.

1.2 Personal Computer Requirements

The program that controls the High Pressure Flow Meter is HPFM95NTXP.EXE. To run this software you need an IBM compatible computer with an 800 x 600 pixel screen or better. The computer must be running on Microsoft Windows 95, 98 or ME with Version 1.09 and it must have a parallel printer port. The Version 2.12 will also work with Windows NT, 2000 or XP. We suggest that you use a notebook or sub notebook computer with the HPFM, especially if you plan to use the HPFM in the field.

The software is installed from a single CD ROM via an Install Shield Wizard in the same manner as most Windows software. The install shield Wizard will install the program in:
C:\Program files\Dynamax\HPFM95-XPV1.12 (or similar name as later versions are released by the USDA Forest Service, c/o Mel Tyree). The Wizard will add a HPFM icon to your desktop. The icon looks like an 8-way valve.

Five steps to install HPFM software:

1. Install Port95nt.exe from your CD ROM. This step is only performed if you are running a Windows 2000, NT or XP on your PC. Otherwise, skip to step 3.
2. Restart PC as prompt.
3. Install HPFM by running setup.exe from your CD ROM.
4. Copy HPFM.cal from your CD ROM under folder name “calibration” to C:\Program files\Dynamax\HPFM95-XPV1.12, it will replace the old HPFM.cal file.
5. An 8-way valve icon is created automatically on desktop and can be used to access program directly.

Dynamax supply the HPFM with a professional factory calibration. The calibration file is included on the software disk under the file name HPFM.CAL. If you wish to perform a custom calibration of the HPFM, you will require an Electronic Balance with a resolution to 4 decimal places and RS232 communication facility both on the balance and the computer. However, it is recommended that Dynamax perform all calibrations.

1.3 Pressured Bottle Requirements

Dynamax ships the HPFM-C models with a compressed air tank, regulator, and hose for connection to the HPFM. This tank can be pressured to 2015 pounds per square inch (PSI) or 13,890 Kilo-Pascal’s (kPa). It has a volume of 24 cubic feet (Cu. Ft.) or 682 liters. The regulator allows control of pressures up to 1,500 PSI or 10,343 kPa. This air tank or bottle cannot be shipped filled, so you will need to fill the bottle (air tank) with a **dry** compressed gas. Economically, two gases are best: Nitrogen or dried compressed air. The compressed air tank can be filled by University physical plants (they can assist you) or distributors such as Air Liquide, Linde, or BOC Gases.

WARNING!

The compressed air tank must have at least 700 PSI (4,800 kPa) to run a transient pressure test.

The setup for running a transitory test requires enough pressure (600 PSI or 4,100kPa) to deliver a constant flow of gas to the HPFM. The pressure regulator requires about 700 PSI in the tank to deliver 600 PSI to the HPFM. It must deliver the gas without "choking" and causing a reducing flow of gas over the time period of the test.

1.4 Unpacking

The HPFM is supplied as a complete unit. No assembly is required. However, there are several accessories that are packaged with the system such as:

- **O-rings**
- **Compression caps**
- **Connection and bleeding hoses**
- **Compression Couplings**
- **Battery Charger**
- **Communications Cable**
- **Manual & Software**

It is recommended that you take a few minutes to check off and confirm all of these components were received. A detailed Parts and Packing List is included in Appendix I.

1.5 What to Keep!

NOTE

When unpacking, do not discard the box and packing material.

Dynamax ships the HPFM in a box with material on all six sides. This makes the shipping box the perfect box for you to ship your HPFM internationally, across the U.S., or back to Dynamax for an annual calibration. Also keep the Lists of Parts, the software, and the tools that came with the HPFM. The list will come in handy when reordering parts for the HPFM. The software should be kept as a backup in case of PC failure such as hard drive corruption. The tools, which can be found in the lid of the HPFM, should be kept there since they will be needed to use the HPFM on a daily basis.

2. Quick Start Guide

This section is designed to provide a recipe style reference guide to present the basic steps you should follow to assist the new user to begin using the HPFM. The manual then provides more detailed instruction on how to complete each step.

1. Fill the Air Supply Tank with Compressed Air or Nitrogen – **Section 4.3**

2. Prepare 7.6L (2 gallons) of degassed de-ionized water – Section **4.4.1**

3. Prime the Captive Air Tank of the HPFM with degassed de-ionized water – Section **4.4**

4. Eliminate all air from the HPFM by purging air from the CAT and Tygon supply tubing, through the purge valve and water filter purge caps. Then bleed the system with the small **Red** capillary tube over night to remove any microscopic air bubbles that may have remained in the CAT or the 8-Way valve manifolds. – Section **4.5**

5. Install the HPFM software on your PC and connect the HPFM to a PC via the computer's Printer Port (LPT1) and the communication port on the HPFM with the communications cable. – Section **5.1**

6. Turn HPFM on and launch the program by clicking on the HPFM95 icon – Section **5.3**

7. Set the Zero Flow Volts to zero the transducers before taking measurements. This must be performed each day before beginning measurements to ensure accuracy – Section **5.4**

8. Select the plant that you will be working with and measure the stem size – Section **7.0**

9. Cut the sample plants stem and install the Pressure couplings – Section **7.0**
   Use (1 mm ID) HPLC "Natural" (light tan) tubing with fittings 1-20 mm (HPCC 1-4, HPCC 4-10 and HPCC 10-20) and (1.5 mm ID) Teflon HPLC "Clear" tubing with fittings 19-50 mm (HPCC 19-35 and HPCC 34-50)

10. Determine the correct flow range to use for your selected sample. If you do not have the correct flow range set your measurements will be over-scale and you may think the system is not functioning properly. – Section **5.5**

11. For roots begin your experiment with a Transient Measurement function, as this is the quickest method for gathering hydraulic conductance data and will minimize any plugging effects caused by the plants natural healing process. For large stems you may need other methods – Section **5.6**

12. After collecting transient flow data you can immediately perform a Regression analysis of the data using the HPFM software to determine the Hydraulic Conductance of the sample plant. Record the temperature of the test in a comment field. – Section **5.6.2**

13. As your understanding of the system develops, begin using the Quasi-Steady State Flow Meter function. By measuring total resistance of the sample you can determine the

contribution that individual components make to the overall hydraulic conductance of the plant. – Section **5.7**

14. Before beginning an intensive experiment be sure to understand all the necessary tips and cautions required to measure hydraulic conductance successfully – Section **6.0**

3. Application and Theory of Operation

3.1 Why use the HPFM?

The High Pressure Flow Meter has several uses in the analysis of root/shoot studies. Some examples of the uses of the HPFM are:

- **Root conductance in the lab or field.**
- **Conductance of shoots and petioles with or without leaves.**
- **Root Stress Analysis on trees or crops.**
- **Modeling root to shoot communications.**
- **Transpiration models.**
- **Root water status studies.**
- **Absolute varietal comparison statistics.**
- **Mycorrhizae nutrient/water enhancement studies.**
- **Soil to root conductance statistics.**
- **Crop conductance studies.**

3.2 Transient Measurements Conductance of Roots

Experience has shown that during flow measurements on roots, flow frequently declines even when the applied pressure, $\mathbf{P_2}$, is constant. Reasons for this include:

1. During perfusion, solutes accumulate in the stele by reverse osmosis causing a continual decrease in driving force on water movement.

2. Natural wound responses induced when the shoot is excised cause plugging of the xylem within the first few centimeters of the wound causing a continual decline in **K**.

Readers are referred to (Tyree et al. 1993 and Tyree et al. 1995) for details. Consequently, the HPFM has been designed to measure **K** quickly before the above effects become serious enough to influence **K**. But doing rapid measurement raises other sources of error that need to be addressed.

NOTE

It is essential that users of the HPFM are aware of potential sources of error and know of ways to minimize such errors.

The rate of water flow into roots, **F**, will exceed the rate of passage through the roots, $\mathbf{F_h}$, if the root is initially dehydrated since some of the water will remain behind to re-hydrate cells. But **F** will exceed $\mathbf{F_h}$ even in fully hydrated roots because pressurization will cause elastic swelling of the roots or the HPFM. **F** will also exceed $\mathbf{F_h}$ because of compression of any air-bubbles that might be present in the wood, the stem interface, or the HPFM. So **F** will be given by:

$$\mathbf{F = F_h + F_e + F_b} \qquad \mathbf{(5)}$$

Where: F_e is the flow associated with elastic swelling and F_b is the flow to compress air bubbles (when present).

If the flow through the object measured is a linear function of applied pressure difference between the outlet of the HPFM and atmospheric pressure, we can express F_h as:

$$F_h = K P_2 \quad (6)$$

Where: P_2 is the outlet pressure relative to atmospheric pressure. The volume of the tubing, connectors, and object measured will increase with P_2. If the volume increase is elastic and linear with pressure, then the volume of the system, **V**, will be given by:

$$V = V_o + P_2/e \quad (7)$$

Where: V_o is the initial volume of the system at **P2 = 0** and e is the bulk modulus of elasticity. The time derivative of Equation (7) gives the flow to cause the elastic volume change:

$$F_e = dV/dt = (1/e)\, dP_2/dt \quad (8)$$

If air bubbles are present anywhere in the system, they will be compressed according to the ideal gas law as the pressure of the fluid around the bubble increases. If V_b is the volume of a bubble at absolute gas pressure P_b then the ideal gas law gives:

$$P_b V_b = nRT = P_i V_i \quad (9)$$

Where: n is the number of moles of gas in the bubble, **R** is the gas constant, T is the Kelvin temperature, and P_i is the initial gas pressure and V_i is the initial volume. If we write Equation (9) as $V_b = V_i P_i / P_b$ and take the derivative with time we get the rate of volume change of the bubble:

$$dV_b/dt = -(V_i P_i / P_b^2) dP_b/dt \quad (10)$$

The negative sign of the derivative indicates that V_b decreases with increasing P_b. The flow of water to compress the gas volume is the negative of Equation (10) so we have:

$$F_b = -dV_b/dt = (V_i P_i / P_b^2) dP_b/dt \quad (11)$$

In the case where all of the air bubbles are near the outlet side of the flow meter, we can equate P_b **to** $P_2 + P_i$. P_i is the initial absolute pressure of the bubble when $P_2 = 0$. P_i approximately equals 0.1 MPa. The barometric pressure will determine the **Pi**. There will also be a small contribution by the surface tension of the air-water interface to bubble compression. In the special case where the air bubbles are near the outlet of the flow meter, the dynamic flow will be given by substituting Equations (6, 8 and 11) into (5):

$$F = K P_2 + (1/e)\, dP_2/dt + V_i P_i / (P_2 + 0.1)^2\, dP_2/dt \quad (12)$$

During normal operation of the HPFM, the inlet pressure P_1 increases linearly with time, and after a short time delay this causes P_2 to increase linearly with time to make dP_2/dt equal to a constant. This permits easier interpretation of results because it makes the elastic contribution a constant offset to F_h. The contribution of the three terms in Equation (12) is illustrated in

Figure 2 for the case where $\mathbf{P_2}$ increases linearly with time from time = 0 (Figure 2 (A)). The component flows **(F_h, F_e, and F_b)** and total flows are shown in Figure 2 (B).
It is not possible, in practice, to make $\mathbf{P_2}$ increase linearly with time from time 0. This is because an extra conductance equal to the conductance of the capillary tubes of the HPFM **(K_{CT})** is interposed between the pressure transducers that measure $\mathbf{P_1}$ and $\mathbf{P_2}$ (see Figure 1.). When the full set of equations is derived (not shown) a short time lag is predicted before $\mathbf{P_2}$ increases linearly with time (see Figure 2 (C)). The resulting component and total flows are shown in Figure 2 (D).

Air bubbles generally can be avoided within the flow meter tubing and apparatus, but sometimes air is in the vessels and/or intercellular air spaces in the base of roots. Although the compression of air causes an increase in **F** at any given $\mathbf{P_2}$ during a transient measurement, bubbles cause an underestimation of the root conductance, $\mathbf{K_r}$. This is because $\mathbf{F_b}$ decreases with increasing $\mathbf{P_2}$ causing a negative contribution to the slope used to calculate $\mathbf{K_r}$. Fortunately, the contribution of bubble compression to total flow diminishes with increasing pressure. The slope of the **F** versus $\mathbf{P_2}$ for $\mathbf{P_2 > 0.25}$ **MPa** is a reasonable approximation of the hydraulic conductance of the root system being measured. It is probably good practice to measure transient flows for $\mathbf{P_2}$ up to **0.5 MPa** in order to reduce the underestimation of $\mathbf{K_r}$ caused by compression of air bubbles.

3.3 Quasi-steady State Measurements of Hydraulic Resistance, R_{QS}

During steady-state measurements of $\mathbf{R_{QS}}$, water flow and applied pressure are both constant and by definition the water flow into the object measured equals the flow out of the object. In practice it is never possible to keep flow and pressure perfectly constant, so it is best to refer to such measurements as quasi-steady state.

The capillary tubes (CT) on the HPFM have been calibrated, i.e., their conductance, $\mathbf{K_{CT}}$, or resistance $\mathbf{R_{CT} = 1/K_{CT}}$, has been measured. During flow measurements, the control program, (HPFM95.EXE) monitors the difference in pressure **dP** across the CT. The program uses **dP** to calculate the flow, **F**, from:

$$\mathbf{F = K_{CT}\, dP} \qquad \mathbf{(1A)}$$

or

$$\mathbf{F = dP/R_{CT}} \qquad \mathbf{(1B)}$$

Since the flow into the object closely equals the flow out, the unknown hydraulic resistance, R_U, can be calculated from:

$$\mathbf{R_U = (P_2 - P_O)/F} \qquad \mathbf{(2A)}$$

or

$$\mathbf{K_U = F/(P_2 - P_O)} \qquad \mathbf{(2B)}$$

Where: $\mathbf{P_2}$ is the pressure recorded by the pressure transducer at the outlet, PT2, and $\mathbf{P_O}$ is the pressure of the water where it emerges from the object. In many cases $\mathbf{P_O}$ is known to be zero. When $\mathbf{P_O}$ is unknown then $\mathbf{R_U}$ or $\mathbf{K_U}$ cannot be computed. Examples will be given later of instances where $\mathbf{P_O}$ is unknown. For the rest of this section we will assume $\mathbf{P_O = 0}$ and can be omitted from the equations.

Another useful way of viewing Quasi-steady state measurements is through the Ohm's law analogue for flow through resistance in series. For any given resistance, **R**, in series Ohm's law states that:

$$\mathbf{RF = dP} \qquad \mathbf{(3)}$$

So for the capillary tube in series with the unknown resistance we have:

$$\mathbf{R_{CT}\, F = dP} \qquad \mathbf{(4A)}$$

and $$\mathbf{R_U\, F = P_2} \qquad \mathbf{(4B)}$$

So dividing Equation (4B) by (4A) we have:

$$\mathbf{R_U/R_{CT} = P_2/dP} \qquad \mathbf{(4C)}$$

or $$\mathbf{R_U = R_{CT}\, P_2/dP} \qquad \mathbf{(4D)}$$

or $$\mathbf{K_U = K_{CT}\, dP/P_2} \qquad \mathbf{(4E)}$$

4. Setup for the HPFM

4.1 Operational Imperative

From the start, it is imperative that users of the HPFM take the following steps:

1. Always test for air bubbles in the HPFM prior to starting experiments. See **Subsection 4.5**, "*Eliminating Air from the HPFM*" on use of software for methods of testing for air bubbles. Please read **Subsection 6**, "*Tips and Cautions*" as well.

2. Connections between the **Purple** outlet of the 8WO and the compression fitting on the object must be checked. In order to reduce elastic flow, F_e, always use small-diameter, rigid outlet tubing when using the two lowest flow rate ranges (**Green** and **Orange**). For high flow ranges (**Red**, **Yellow**, **Gray** and **Brown**) you may use the clear 1.5 mm ID Teflon FEP "Clear" tubing supplied by Dynamax. For low flow ranges (**Green** and **Orange**) ONLY use the 1.0 mm ID "Natural" (light tan) HPLC tubing supplied by Dynamax for connections between the purple outlet of the 8WO and the compression fitting on the object to be measured.

NOTE

DO NOT USE 0.12 mm ID RED TUBING FOR CONNECTION BETWEEN THE HPFM AND A PLANT SAMPLE!

The red tube is used only for testing and bubble removal (see sections 2.4 and 4.5)

3. Carefully follow the instructions in **Section 4.4**.

4.2 Valve Setting for Preparing the HPFM for Service

The valve settings are important in removing air bubbles (gas) and keeping the air from infiltrating the system at different points in the handling, use, and storage of the HPFM. During the storage of the HPFM unit make sure that the purple outlet value is closed.

4.3 Pressurizing the HPFM

The HPFM uses a pressure bottle filled with nitrogen or air (your choice) to force water into the root, stem, or shoot. This pressure bottle can be filled to a pressure of 15 MPa (2,200 PSI). The bottle is shipped empty for safety reasons. To fill the bottle, contact your local gas distributor or physical plant. Depending on your preference or economics, the bottle can be filled with either air or nitrogen.

Gauges Specification on the regulator:

Supplying Pressure Display – 4000 PSI (Max)
Delivery Pressure Display – 1000 PSI (Max)

The bottle also has a pressure regulator that is attached to the top of the bottle using a screw clamp. Follow the directions enclosed for attaching the regulator to the bottle correctly. Use soapy water or a commercial product such as "Snoop" to check for leaks not only in the regulator-to-bottle connection, but the other hose connections as well.

4.3.1 Tips for Setting the Pressure Regulator

The valve at the top of the bottle should not be opened until the regulator pressure set screw is backed off completely. Once this is done, open the valve on the bottle completely. Check the bottle pressure gage. The pressure should be at least 4.15 Mpa (600 PSI). This pressure is required when running a transient state flow. The minimum pressure forces enough compressed gas to the captive air tank (CAT) to keep a steady, linear pressure increase during the test. Close the tank valve, and release the hose pressure by pressing on the valve release needle.

WARNING!

Do not pressure the compressed air supply hose to above 689 kPa (100 PSI) while the hose is disconnected!
Do not disconnect or connect the hose if the pressure is above 70 kPa (10 PSI)!

The "disconnects" on the air pressure hose and HPFM system has a working pressure of 689 kPa (100 PSI). Pressure on the disconnect seals above 689 kPa (100 PSI) could cause a catastrophic failure of the "disconnect system" or personal injury. You also can generate a loud, annoying "pop" sound unless the pressure is released first.

Increase the air regulator pressure to 138 kPa (20 PSI) and check for leaks again. Decrease the pressure by backing off the regulator screw. Depress the end of the "disconnect" into a hard flat surface such as a coin. This will open the disconnect seal and release the small amount of gas in the hose.

Photo 1
Air Regulator Pressure

4.3.2 Preparing the Compressed Air Supply System for use with the HPFM

Connect the hose quick-disconnect (Male) into the HPFM compressed air supply quick-disconnect (Female). Make sure that the needle valve is opened about 4 turns. The airflow control valve (AV) should be closed or pointed directly up. Remember that turning the valve to the right will bleed pressure from the HPFM. Turning the valve to the left will increase pressure when the compressed air supply is connected. If you are setting up to measure a quasi-steady state flow, turn the Needle Valve (NV) next to AV until the digital gage reads ~550 kPa (~80 PSI). Do not go over 550 kPa (~80 PSI). The HPFM has a safety relief valve which is set for ~650 kPa (~95 PSI).

Photo 2 Compressed air and HPFM near connection

WARNING!

Do not attempt to adjust or "plug" the safety relief valve!

If you wish to test the unit or run a quasi-steady state flow test, open the Compressed Air Valve until there is a steady increase in the pressure in the CAT. Fill the CAT at a rate of about 7-10 kPa (1-1.5 PSI) per second. The Needle Valve can be used to regulate the rate of increasing pressure. Close the Compressed Air Valve (AV in Photo 4 page 23) when you have reached the required pressure in a range from 200 to 500 kPa (29 to 72 PSI).

If you are doing a transient pressure test, release CAT pressure at 0 to 7 kPa (0 to 1 PSI) and turn the pressure regulator screw until the outlet pressure is at least 4.1 Mpa (600 PSI). Make sure that the needle valve is opened about 2-3 turns so that the pressure increases about 7kPa (1 PSI) per second. Open Compressed Air Valve only in conjunction to starting the software for the test.

4.4 Adding De-gassed Water to the HPFM

The Captive Air Tank (CAT) is a two-compartment tank. The upper compartment contains water and the lower section can be pressurized with air. A flexible rubber diaphragm separates the two compartments. The large stainless steel CAT allows you to pressurize the water without the air and water mixing together. The CAT will hold 7.0 liters of water and it is best to refill while there is still at least 0.5 liters of water in the tank. If the CAT is initially empty then refer to Subsection 4.4.3, "*Priming a totally empty tank*".

Normal tap water or water that has been standing naturally absorbs air. The particles in naturally found water and/or tap water plug the very small inner diameters of the tubing used in HPFM as well.

CAUTION!

Always use Distilled Degassed Water in the HPFM.

If the dissolved air effervesces, or the air comes out of solution (much in the same way a soda pop will effervesce when the can is opened), then air bubbles will form. These air bubbles will compress as the pressure increases. However, when the water is flowing from the HPFM and the pressure decreases, the air can come out of the solution. Air and water behave differently when compressed, or pressurized. Water, for the most part does not compress, with its volume changing little. Air will compress with great changes in the volume that the air occupies. That large change is why air must be removed from the HPFM system.

The water added must have the air removed. There are two accepted ways for removing the absorbed air from water. The easiest way is to "boil" the water or raise the temperature of the water to the vapor point of water 100 ^{o}C (212 ^{o}F). However boiling removes less than half the air. A more effective way is place the water in a vacuum. Degassing the distilled water is

explained in **Subsection 4.4.1**, "*Making Degassed Water / Preventing Algae Buildup Inside the HPFM*".

Your HPFM system comes with a HPFM refill kit. The refill kit is a 7.6 L (2 gallon) tank that can be pressurized by pumping air with the handle on the top of the unit. This pressurization will force the degassed water into the CAT of the HPFM when connected. The Refill Kit tank conveniently allows refilling of the HPFM just about anywhere. There are a few important things to remember about the HPFM. The first is to always visually check the level of the degassed water in the HPFM Refill Kit. The degassed water level should never go below the siphon point inside the Refill Kit tank. If air were introduced into the HPFM system during filling, then this would mean removing the air and follow the procedures in **Section 4.5**, "*Eliminating Air from the HPFM*".

Caution: If degassed water is kept in the refill container more than a few minutes under pressure it will become supersaturated with air again. So fill immediately after degassing!

Photo 3
Refill Kit

4.4.1 Making Degassed Water / Preventing Algae Buildup Inside the HPFM

The Captive Air Tank (CAT) should be filled only with 'degassed water', i.e., water with air dissolved at less than saturating concentrations of O_2 and N_2 at atmospheric pressure. There are two methods of making degassed water. The first method is to boil water. This removes some of the absorbed gas because air is less soluble in hot water than cold water. If this method is used, the CAT could be filled while the water is still hot.

WARNING!

Water Degassed by boiling will be hot!
Take precautions when handling the boiling hot water!
Use insulated gloves for handling hot material.

The steps for boiling the distilled water for the HPFM are:

1. Start off with a 3.8 L (~1 gallon) of distilled water. Distilled Water is convenient and easy to obtain. It is even available in most food markets.

2. Boil the distilled water for 5 minutes.

3. Let the distilled boiled water stand for 10-15 minutes before handling and adding the water to the refill kit.

4. Add one drop of algaecide (from the HPFM accessories kit) to the distilled water.

5. If the HPFM is new, it will be shipped empty. Repeat steps 1 through 4 so that you will have 7.6 L (2 gallons) of water to fill the CAT.

6. If the HPFM is new or been shipped, proceed to **Subsection 4.4.2,** "*Priming a totally empty CAT*".

The other method is to put water into a vacuum flask and draw a vacuum over the water for 5 to 10 minutes. The steps for this procedure are:

1. Place the distilled water in a vacuum flask. Fill the flask to its logical level. Do not over fill. Distilled Water is convenient and easy to obtain. It is even available in most food markets.

2. Vacuum the distilled water for 5 minutes from the time that the water begins to effervesce with air bubbles or "boil" at room temperature. If the vacuum is really good you should see the water boil at room temperature when the pressure drops below about 4 kPa (0.6 PSI) absolute.

3. Pour the newly degassed water into a secondary container. Repeat steps 1 and 2 until one gallon of degassed water has been produced.

4. Add one drop of algaecide (from the HPFM accessories kit) to the distilled water.

5. If the HPFM is new, it will be shipped empty. Repeat steps 1 through 4 so that you will have 7.6 L (2 gallons) of water to fill the CAT.

6. Fill the Refill Kit immediately and close the Refill Tank.

7. If the HPFM is new or been shipped, proceed to **Subsection 4.4.2,** "*Priming a Totally Empty CAT*". The tank should be filled immediately after degassing the water by vacuum. Use the Refill Kit to insert the degassed water into the Captive Air Tank of the HPFM as quickly as possible.

4.4.2 Priming a Totally Empty Captive Air Tank (CAT)

When first priming the HPFM or if air bubbles have entered into the HPFM system, you must prefill the CAT with at least 2 liters of degassed, distilled water before you can start the priming process. If the HPFM has had water already added, please skip this subsection and move on to **Subsection 4.4.3**, "*Adding water to a Partially Empty Captive Air Tank (CAT)".* This pre-fill will assist in the removal of all air bubbles. Removing air bubbles as in **Section 4.5**, "*Eliminating Air from the HPFM*" is a requirement for normal operation of the HPFM. Another requirement is the removal of small microscopic bubbles that form inside the wetted areas of the HPFM and adhere to the surfaces. Note that parts of the series of instructions are very much like **Subsection 4.4.3**. That is because you must fill the HPFM unit with water to remove the air bubbles first. The steps for priming the empty HPFM CAT are:

1. Make sure that all valves are closed.

2. Turn on the digital pressure gage. If there is pressure, begin to release the pressure with the Compressed Air Supply Valve. To release pressure, slowly turn the valve to the bleed air position (right).

3. Once the pressure is removed, close the Compressed Air Supply Valve.

4. Connect the Refill Kit to the HPFM using the following steps:

   a. Make sure that there is degassed water in the Refill Kit. If not, go to **Subsection 4.4.1**, "*Making Degassed Water*". Make sure that you have enough water to finish the sampling project in the Refill Kit, with water to spare. Some of the water will be used for remove extraneous bubbles from the tubing, root compression fittings, etc.

   b. Pump the Refill Kit with several hand strokes. This is the pressure that will force the degassed water into the HPFM.

   c. Raise the refill tubing straight upward. Depress the Refill Kit male "disconnect" against a hard object, such as a flat blade screwdriver, allowing the water to flow and remove bubbles from the Refill Kit tubing. **Cover the "disconnect" and screwdriver with a towel to keep the hot water from spraying.**

WARNING!

Take Precautions. Use Gloves when handling the Hot Degassed Water.

   d. Connect the Refill Kit to the HPFM check the tubing for air bubbles and leaks. Open the Water Supply Valve to the left to allow the water to flow into the CAT.

   e. Pump up the Refill Kit every 1-L (0.25 gallons) with another 10-15 strokes, keeping pressure and water flow into the CAT. Note the digital pressure gage will show an increasing pressure on the compressed air supply side of the HPFM.

   f. When and the digital pressure gage reaches 15 kPa (2-3 PSI), turn the Compressed Air Supply valve to bleed the pressure from the HPFM System.

   g. Make sure that you do not allow air from the Refill Kit tank to enter into the HPFM. Stop when you have about 1 liter (1 quart) of water left in the refill kit.

   h. Once the HPFM is filled, close the Water Supply Valve.

5. Disconnect the water connection from the Refill Kit.

6. Pull the pressure release on the side of the Refill Tank. Lock the Refill Tank Handle by pushing down on the handle with tabs on the handle sliding into the keyed position on the top cap of the tank. Once the handle is completely down, twist the handle. The tank handle should now be locked in position.

7. You will now need to remove any air from the HPFM system. This can be accomplished by referring to **Section 4.5**, "Eliminating Air from the HPFM".

4.4.3 Adding Water to a Partly Empty Captive Air Tank (CAT)

It is important to prevent air bubbles from entering the Captive Air Tank (CAT) because these bubbles will re-saturate the degassed water. See **Section 1,** "*High Pressure Flow Meter (HPFM) Form & Function*" in this manual to learn why it is important that the water be degassed. This section assumes that the HPFM has been used (and/or filled) before. If the HPFM has not been used or air has been allowed into the HPFM Captive Air Tank (CAT), Filter, or 8 Way Manifold systems, then begin with **Subsection 4.4.2**, "*Priming a Totally Empty Captive Air Tank (CAT)*".

Follow these steps:

1. Make sure that all valves are closed.

2. Turn on the digital pressure gage. If there is pressure, begin to release the pressure with the Compressed Air Supply Valve. To release pressure, slowly turn the valve to the right, bleeding the air.

3. Once the pressure is removed, close the Compressed Air Supply Valve.

4. Connect the Refill Kit to the HPFM using the following steps:

   a. Make sure that there is degassed water in the Refill Kit. If not, go to **Subsection 4.4.1**, "*Making Degassed Water Preventing Algae Buildup Inside the HPFM*". Make sure that you have enough water to finish the sampling project in the Refill Kit, with water to spare. Some of the water will be used for remove extraneous bubbles from the tubing, root compression fittings, etc.

   b. Pump the Refill Kit with ten to fifteen hand strokes. This is the pressure that will force the degassed water into the HPFM.

   c. Raise the "disconnect" of the Refill Kit straight up and cover the "disconnect" with a towel or pads designed for hot Liquids. This will prevent hot water from spraying.

   d. Depress the Refill Kit male disconnect against a hard object, allowing the air to flow out and remove bubbles from the Refill Kit tubing.

   e. Connect the Refill Kit to the HPFM and check the tubing for air bubbles and leaks. Open the Water Supply Valve to the left to allow the water to flow into the CAT.

f. Pump up the Refill Kit every 1 L (0.25 gallon) with another 10-15 strokes, keeping pressure and water flow into the CAT. **Note**: the digital pressure gage will show an increasing pressure on the compressed air supply side of the HPFM.

g. When the digital pressure gage reaches 15 kPa (2-3 PSI), turn the Compressed Air Supply valve to bleed the pressure from the HPFM System.

h. Make sure that you do not allow air from the Refill Kit tank to enter into the HPFM.

i. Once the HPFM is filled, close the Water Supply Valve.

5. Disconnect the water connection from the Refill Kit.

6. Look closely for any air bubbles in the tubing lines within the HPFM unit. If you find any, refer to **Section 4.5**, "*Eliminating Air from the HPFM*".

7. The HPFM unit should now be primed and ready to use.

NOTE: The best way to avoid risks of burns when filling the HPFM with degassed water is to degas water with a vacuum pump at room temperature. A vacuum pump adequate for the job should cost about $500. An alternative filling method recommended by Mel Tyree is to make vacuum degassed water in a 10-liter thick-walled glass carboy. The HPFM can be filled directly from the carboy to the CAT. The carboy is placed on top of the HPFM and length of tygon tubing is placed in the carboy and primed with water and then connected to the OQ removed from the Refill Kit. Connect the OQ while the CAT is under 40 kPa (6PSI) of pressure to expel any air bubbles into the carboy then release the pressure. The CAT will refill under gravity. **THE LESS YOU MANIUPLATE THE WATER AFTER DEGASSING THE LESS UNWANTED GAS YOU WILL HAVE IN THE HPFM.**

4.5 Eliminating Air from the HPFM

Before eliminating air from the HPFM for the first time, study Photo 4, which gives the location of components on the HPFM. The labels are:

AV = Air Valve

NV = Needle Valve

CAT = Captive Air Tank

PS = A/D Circuit Power Switch

GQ = Green quick fit connector for adding air to CAT

OQ = Orange quick fit connector for adding water to CAT

PT1 = Pressure Transducer #1 on inlet side

PT2 = Pressure Transducer #2 on outlet side

8WI = 8-Way Inlet Manifold

8WO = 8-Way Outlet Manifold

DP = Digital Pressure Gauge

PR = Pressure Regulator (Not Shown)

PV = Purge Valve (Not Shown)

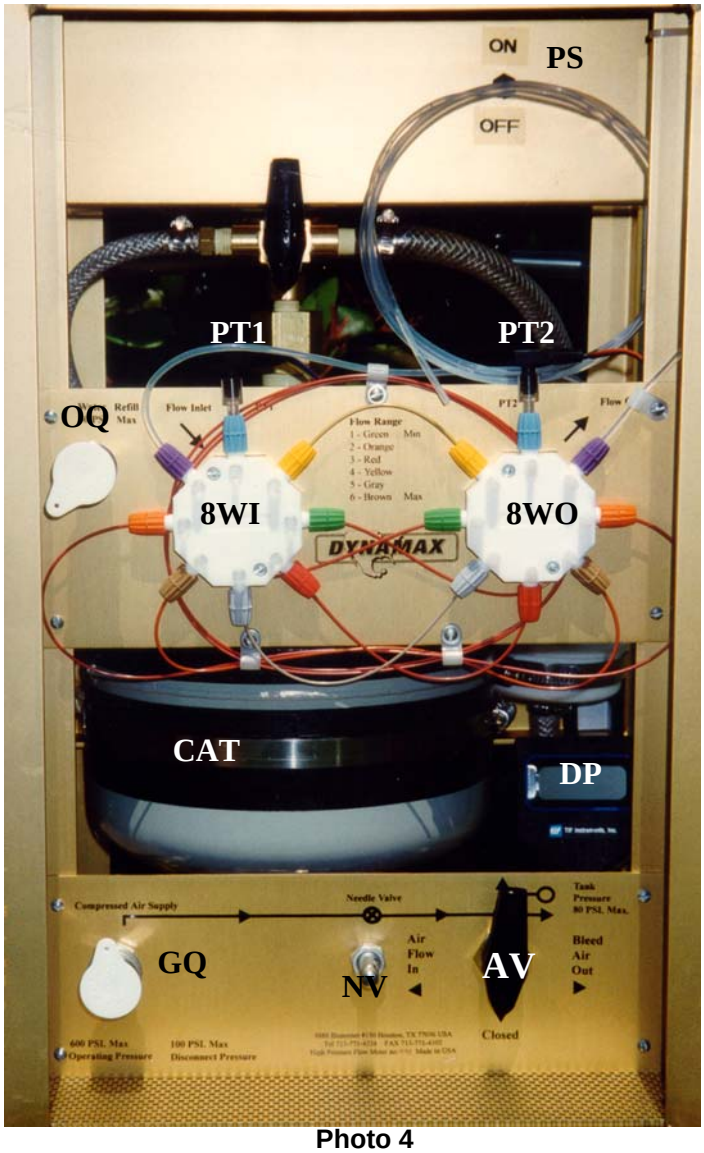


Photo 4

A lab version with larger stainless steel CAT fills most of the available space in the aluminum frame so the compressed gas tank is external.

The areas of the HPFM that require purging are all internally wetted parts. The goal is to have no air bubbles in the system. The major areas to check and purge are the CAT, the tubing, the 8-way manifold, and the HPFM Refill Kit. The HPFM can have air trapped in the CAT, the valve at the top of the CAT, and the tubing from the CAT to the filter. This can be removed quickly by:

1. Protect the digital pressure gage and be prepared to wipe up and remove excessive water on or around the HPFM. The digital pressure gage can be protected with a small plastic bag placed over the gage and held in place with a rubber band or tie wraps.

CAUTION!

Always protect the Digital Pressure Gage from getting wet. Place a plastic bag over the unit whenever you are adding water or removing air from the system.

2. To facilitate purging of the HPFM there is a purge valve at the top of the CAT. Apply approx. 15 kPa (2 PSI) pressure to the unit and ensures the three-way valve is closed. If a pressure greater than 15 kPa (2 PSI) is applied water will spray from the purge valve under pressure.

3. Using a rag to absorb water, slowly open the valve and allow air to escape from the purge valve. Once all the air has escaped and only water flows from the valve close it firmly. **Note:** Tilting the HPFM slightly forward so the purge valve is at the highest point can improve the performance of the valve.

4. If any air bubbles remain in the tubing, exhaust all the air from the system and open the purge valve to allow air back into the valve/tee assembly. Close the three-way valve and pressurize slightly then use the purge valve to try and remove the remaining air.

5. If visible bubbles still remain, one tip is to tilt and/or move the HPFM so that you can see the bubbles. This will also assist in moving the air bubbles from the CAT and valves to the tubing.

6. One way to remove these bubbles is to turn the HPFM unit the way the bubbles move into the water filter. You can then loosen the red cap of the top bleed port to remove the air bubbles. Be careful and only loosen the cap. Re-tighten the cap afterward.

7. If you cannot move the unit, then loosen the hose clamp for tubing at the top of the CAT. Hold the tubing in place while loosening the clamp. Do not let go of the hose. Allow the removal of the air bubbles and while holding the tubing in hand, tighten the hose clamp.

8. Wipe up any excessive water off of the HPFM.

4.5.1 Purging Air from the Water Filter

Water passes from the CAT to the 0.1 μm water filter (F) by way of a 12 mm ID nylon reinforced Tygon tube. If the filter is replaced, air will have to be purged from the new filter. Sometimes during shipping or because of improper filling of the CAT, some air may also enter the micro water filter that needs to be purged. Air bubbles in the filter need to be purged. A small bubble trapped in the filter will eventually dissolve, but larger bubbles must be removed by disconnecting the tubing between the outlet and the 8WI and flushing water through the system under low pressure 20-30 kPa (3-5 PSI).

1. Be sure the CAT has been properly filled with water and is free of air bubbles. If the CAT has been improperly filled, or there may be suspicion of air bubbles in the CAT, then follow the instructions in **Subsection 4.4**, "*Adding Degassed Water to the HPFM*".

2. Pressurize the CAT to 15 kPa (2 PSI) and turn the water valve (WV) from "off" to "flow", i.e., turn the handle clockwise from the vertical position to the horizontal. This should put the water in the filter under pressure.

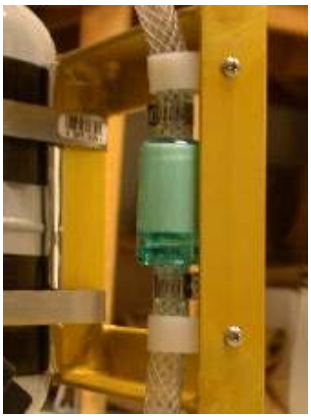


Photo 5
Micro Water Filter

3. Open the Purge Valve (PV) and allow air and water to escape from upper half of the filter and PVC tubing. You may need to tilt the HPFM so the purge valve opening is higher than the rest of the system. After all air has escaped and only water flows out, tighten the purge valve. Then, open the flow inlet to allow rest air inside filter to escape through 8-way valves.

4. If an air bubble appears between the output of the filter and reducing bushing for the Teflon tubing, slightly loosen the 3 mm (1/8") compression nut and allow air to escape. When only water flows, retighten the nut. This should not normally be necessary.

4.5.2 Removing Air Trapped in the 8-way Manifold

Small air bubbles trapped in the 8-way manifolds or in the outlet tubing can seriously degrade the performance of the HPFM during transient measurements of hydraulic conductance (Tyree *et al.* 1995). Air bubbles can be detected by doing a transient conductance measurement with a solid metal plug mounted in the compression fitting. See the instructions under **Section 5.6**, "*Transient Measurement of Conductance*", to see how to perform a transient conductance measurement.

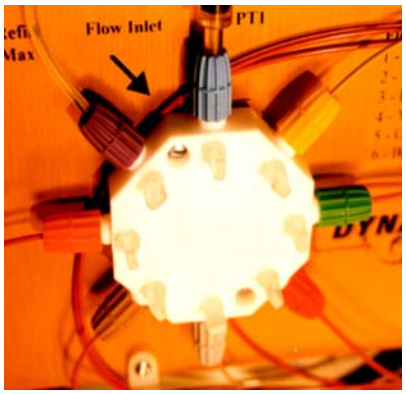


Photo 6
8-Way Valve Manifold

Air bubbles cause errors mostly when measuring low conductance by the transient method when using the lowest three flow ranges, i.e., the **Green**, **Orange** and **Red** ranges. The best way to remove air bubbles from the 8-Way valves is to perfuse water through the system overnight (12 hours) after bleeding the manifold. Steps for perfusing the water can be found in **Subsection 4.4.2**, "*Priming a totally Empty Captive Air Tank (CAT)".* The steps for bleeding the manifold are:

a. Open or make sure to open the Water Supply Valve to the "flow" position (right).

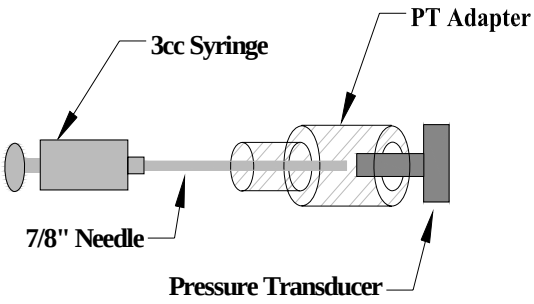
b. Make sure that you still have pressure by checking the digital pressure gage.

c. Open the flow inlet valve. Keep all other valves closed.

d. Loosen connector for PT1 and PT2, allowing a small amount of water to flow out.

e. Close the Water Supply valve removing the pressure from the connection.

Each HPFM is shipped with a 5' (1.5 m) length of **Red** tubing about 0.005" ID. Connect the 1.5 m **Red** tube to the outlet tube using the smallest "Omnifit" connector part. Pressurize the CAT to about 350 – 480 kPa (50-70 PSI) and open all valves on both 8-way connectors. Allow degassed water from the CAT to flow through the system for 8-16 hours. Less than 0.5 liters of water will pass through the HPFM overnight, but that flow is usually sufficient to dissolve all air bubbles trapped in the 8-way valves and the outlet tube. In some rare cases, an air bubble may be trapped in PT2 between the 8WO and PT2. Air must be removed by carefully disconnecting **Blue** caps from the 8WO and injecting water using a **7/8"** hypodermic needle and syringe.



Short Needle Injection Demonstration

4.5.3 Removing Microscopic Air Bubbles from Pressure Transducers

Use the 3cc syringe and 7/8" needle we provided in the accessory kit. Fill it with Algaecide contained water. Eliminate all air bubble inside the syringe and fill the water into Transducers. Keep tapping the adapter on the transducer to move the air bubble to the opening of the Transducer then add water to push air bubble out. **Make sure** you **DO NOT** use the big 1 3/8" stainless steel needle on this procedure or it may damage your Pressure Transducers.

CAUTION!

Be very careful to use 7/8" needle and not to push it all the way to the bottom of the transducers. Failure to do so could cause damage.

4.5.4 Removing Microscopic Air Bubbles from the HPFM - Bleeding

After removing the air bubbles that you can see, we must remove the microscopic bubbles as well. Increasing the pressure and forcing water through a small orifice, or small inner diameter tubing accomplishes this. The bubbles are then either removed or are reabsorbed by the water:

a. Increase the pressure on the HPFM with the Compressed Air Supply system to 550 kPa (80 PSI). If you have not done this or need to review this procedure, please see **Section 4.3**, "*Pressurizing the HPFM*".

b. Connect the 1.5 m (5 ft) **Red** HPLC tubing (ID 0.0127 mm / 0.0005") to the Flow- Out Port of the HPFM. Make sure that the Flow-Out Manifold is closed. You may also connect the **Red** HPLC tubing to the Teflon FEP hose that may already be attached to the Flow-Out valve of the Manifold. This can be accomplished with the HPLC Union included with the HPFM accessories.

c. Open the Water Supply Control Valve to the right allowing water from the CAT to flow through the filter and to the manifold. Open all manifold valves except the Flow Outlet valve.

d. Since there will be a small flow of water from the **Red** HPLC tubing, place the end of the **Red** tubing into a container. Open the Flow Outlet Valve on the manifold. Water should begin to flow in a very small quantity, usually a few drops a minute.

e. As always, check for leaks. Let the HPFM flow like this for **12 hours**. Check the digital pressure gage on a regular basis and add pressure if needed.

f. After 12 hours close all manifold valves and the Water Supply valve.

g. Check for small leaks again. Wipe off any excess water from the frame.

h. Finish filling the HPFM CAT by following **Subsection 4.4.3**, "*Adding Water to a Partly Empty Captive Air Tank (CAT)*".

i. Remove the red tubing before making measurements. Install the connecting hose (1 mm ID "Natural" or 1.5 mm ID "Clear") and bleed out bubbles before connecting to a plant coupling. Proceed to **Section 7**.

j. Now, you are ready to use HPFM on a plant.

4.6 Storing the HPFM

Pressurize the CAT to 140-280 kPa (20-40 PSI), shut the air valve (AV) to retain the pressure. Turn the water valve right (WV) to the "flow" position and close the outlet valve on the 8-way outlet valve (8WO). This will prevent entry of air into your system, keep a minimum pressure on connecting hoses, and your HPFM will remain in optimum form, ready for measurements, whenever you need it. **Be sure to turn off the power to the HPFM A/D board and to the digital pressure gauge to save battery power.** Also be sure to shut off the gas supply to the pressure regulator. This will prevent gas loss from your supply tank in case of a slow leak.

CAUTION!

It is recommended that you keep the HPFM pressurized at 140-280 kPa (20 - 40 PSI) with the outlet valve closed at all times when the HPFM is not in use.

a. Open the Water Supply Valve to the “flow” position (Photo 7.)

b. Make sure that you still have pressure by checking the digital pressure gage.

c. Open the flow inlet valve. Close the outlet valve.

d. Open at least PAIR OF VALVES, e.g. gray range values to equalize pressure between PT1 and PT2.

e. Keeping the system under pressure will prevent entry of unwanted air!

Photo 7
Water Supply Valve

5. Software and HPFM Operation

5.1 Software Installation

Install the software program HPFM95NTXP.exe by inserting the CD in the CDROM drive (for example E:\). Follow the install shield prompts and complete the installation. For ease of support it is recommended that you accept the default installation setting directory settings of C:\Program Files\Dynamax\HPFM95V1.11 (or similar version number). Refer to Section 1.2 for a detailed explanation of software installation.

After the software installation is complete use Windows Explorer to copy the Calibration file HPFM.cal and the calibration data file HPFM_CAL_DATA.txt from the CDROM to the program directory C:\Program Files\Dynamax\HPFM95-XPV1.12. The HPFM.cal file contains the calibration data required by the unit to make measurements. The data file contains all the raw calibration data that was used to make the HPFM.cal file. It is good to keep a backup of this on your hard drive even though you will not use this file.

5.2 System Connections

Connect the pressure regulator to the compressed gas tank you are using (usually compressed air or nitrogen). Be sure the air valve (AV) is in the "off" position. Set the supply pressure to approx. 4 MPa (600 PSI). Turn on the digital pressure gauge. If the HPFM has been previously used it should have been left pressurized to 140-280 kPa (20-40 PSI).

Locate the two 8-way valves and note how the valves are opened and closed (See figure 3). Make sure the valves to the two pressure transducers are ALWAYS left open and that the outlet valve is closed except when water flow is desired. Connect the 25-pin cable between the printer port of the computer and 25-pin communications port on the back panel of the HPFM. Turn on the HPFM (power switch on front panel) and click on the HPFM icon turn on the computer.

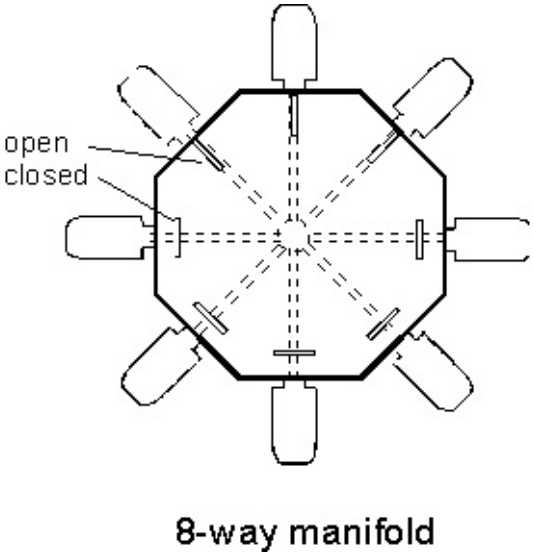


Figure 3
Manifold Openings

To pressurize the system, turn the AV to the fill position (pointing left) and adjust the needle valve (NV) so that the desired rate of pressurization is achieved (normally 0.5 to 2 PSI per second). To release pressure turn the AV to the release position (pointing right). To select a flow range (which is color-coded), open the two valves opposite the two Omnifit connectors with the same color. Make sure all other valves connected to the other capillary tubes are closed.

5.3 HPFM Software Functions

After checking the connection of the communications cable between the HPFM and your PC, turn on the HPFM (power switch on front panel). Double click the HPFM icon. This will bring up the main menu shown below (or something similar):

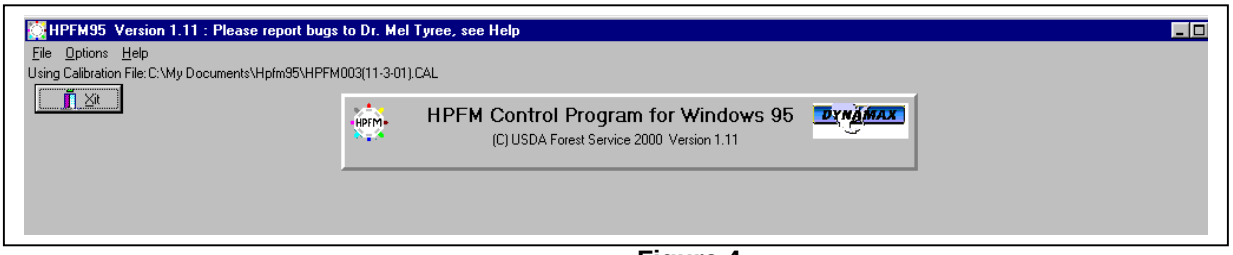

HPFM95 Version 1.11 : Please report bugs to Dr. Mel Tyree, see Help
File Options Help
Using Calibration File: C:\My Documents\Hpfm95\HPFM003(11-3-01).CAL
Xit
HPFM Control Program for Windows 95
(C) USDA Forest Service 2000 Version 1.11

Figure 4
Main HPFM Software Screen

The options in the drop down menus are shown in Table 1 below:

Table 1
HPFM Software Menu options

FILE	OPTIONS	HELP
FileView	Flowmeter	BugReport
XitFileView	Transient	Program Overview
SaveFileView	Regression	COM Ports
View/Set Calib	SetZero	
New CalFile		
Calibrate HPFM		
Exit		

The use of the various main menu items is listed above in the order in which you might use them in the course of a day's work with the HPFM. **Note: the best way to learn the software is to perform the steps below as you read them.**

Figure 5
HPFM Communications Failure Error Message

When the HPFM program starts, the program automatically tests for basic communication with the HPFM by testing to see if the HPFM is connected to the computer through one of three possible printer port connections (LPT1, LPT2, or LPT3). If the HPFM is not found, a warning message is displayed as shown in figure 5. If this message is displayed and you have confirmed the connection and power is correct please refer to **Subsection 10.1** Troubleshooting.

5.4 Setting the Zero Flow Volts

Normally, the difference in pressure (dP) across any given capillary tube should = 0 mV whenever flow is zero at any applied pressure. Although the pressure transducers (PT1 and PT2) are similar, they are slightly mismatched and the degree of mismatch may change slowly with time. The **"SetZero"** option allows you to measure and record the mismatch so that flow measurements can be corrected for the mismatch in order to improve the accuracy of measurements. Thus, it is good practice to perform this test at the beginning of each day you wish to use the HPFM.

The **"SetZero"** option will allow you to do a pressurization of the HPFM with the outlet valve closed and the highest flow range (**Brown**) selected. While pressurization proceeds, you will see a graph of dP (in mV) versus the applied pressure (PT2 in kPa). *If dP exceeds + 2 mV or is less than -2 mV at any time during the test, contact Dynamax or your local distributor for a service adjustment.*

1. Adjust the needle valve so that the rate of pressurization is at the desired rate approximately between 5 to 10 kPa (0.5 to 2 PSI) per second when the AV is in the pressurize position. Then reduce the pressure to < 5 kPa (0.5 PSI) by moving AV to the release position.

2. Select the **Brown** range, i.e., the highest flow range, and make sure the outlet valve is CLOSED.

3. Select **"Options"** - **"SetZero"**.

4. When you are ready to start pressurization, press the start key. As soon as the graph appears on screen, start the pressurization.

5. Continue pressurization. A screen will appear to prompt you to stop when PT2 reaches 550 kPa (80 PSI).

CAUTION!

The computer will tell you when to stop during normal functioning. Do not stop too soon! But if the system malfunctions, do not allow the applied pressure to exceed 690 kPa (100 PSI) as recorded on the digital pressure gage.

WARNING!

Do not allow the applied pressure to exceed 690 kPa (100 PSI) as recorded on the digital pressure gauge. The safety relief valve is set to open at approximately 620 kPa (90 PSI) and close off at approximately 600 kPa (87 PSI). Do not adjust the Safety Relief Valve. Call Dynamax or your distributor if you find a problem or a perceived problem.

6. Stop pressurization when the computer says to and then follow the instructions on the screen to make a regression. You can click on the **"ReGress"** button to do a regression of the curve and you will be given a chance to click on bad points to eliminate from the regression.

7. To save the regression values click on the 'save' button and follow the on screen instructions.

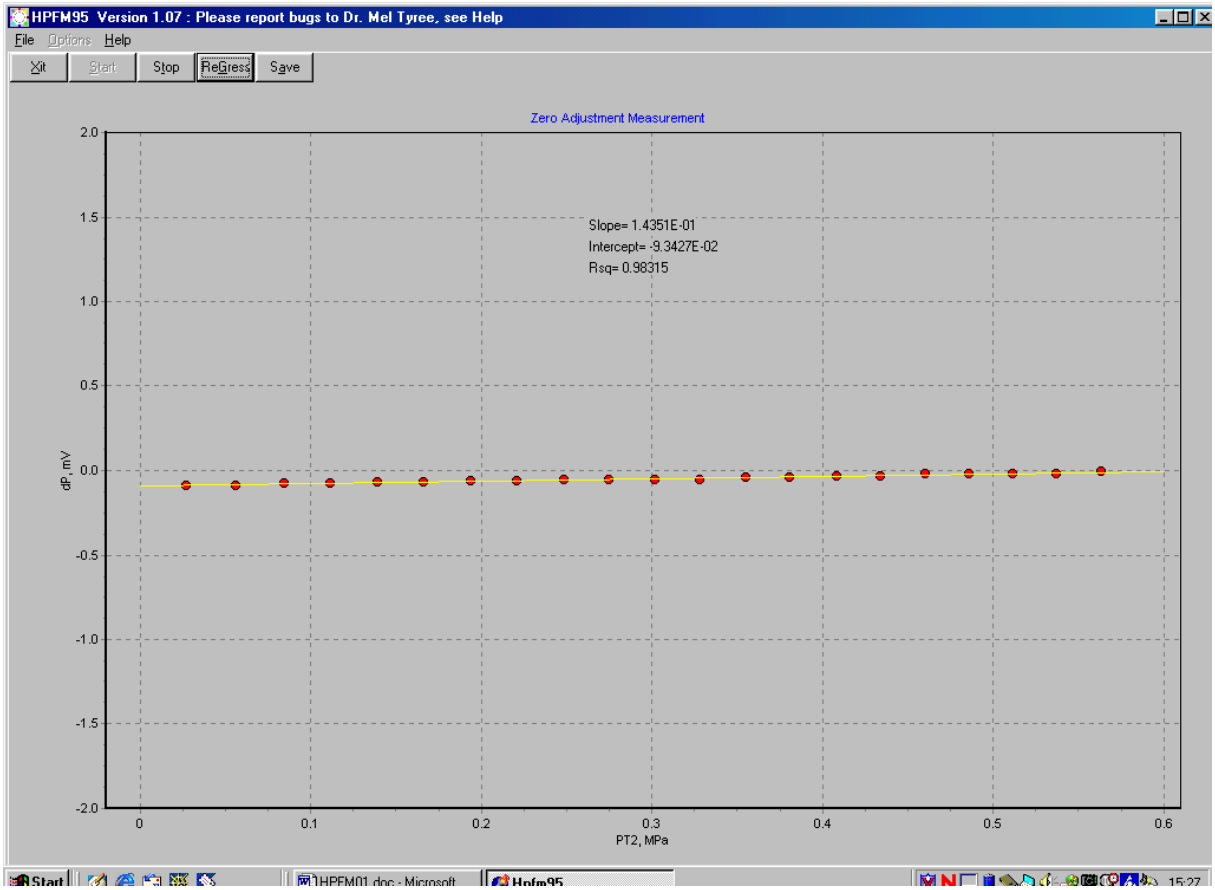


Figure 6

Graphical output of "SetZero" option. X-Axis is PT2 in MPa and Y-axis is dP in mV. Regression slope and R^2 value (Rsq) are displayed at top of graph.

FAQ: When I use software to proceed in “SetZero” procedure, I got all points piled up together at beginning. What do I do?

Answer:

- Check if have all valves on manifolds closed except 2 valves for **Brown** range, 2 valves for pressure transducers and inlet valve.
- Check if you open the water switch by turning in switch to “Water In” position. Third, check if your system’s initial pressure is under 1 Psi = 6.895 kPa.
- Check if you have enough water inside your CAT in order to create a pressure to both transducers. If there is still no response on SetZero, restart your PC and HPFM. If everything is checked and you still receive the same SetZero result, contact Dynamax immediately for assistance.

FAQ: I try to do a “SetZero” procedure, but the system won’t response while I am increasing the pressure. Is there something wrong?

Answer: This is a Common problem.

- Let's check your Calibration data. Please open your HPFM software by double clicking on the icon then go to "File" - "View/Set Calib" to show a screen of your calibration data. Refer to Picture 1 in the attached document. Most of your calibration data is different from this sample so please don't intend to change yours unless instructed. Now, let's take a look at your PT2 values: A0=-1.5219E-03 and A1=6.8965E-03. If you value is different, manually change to the above value by double clicking on them in your "View/Set Calib" screen and type in the correct values. Once it is changed, click on "Save Values" then "Done". If they are the same, just click on "Done" to exit to the main screen.
- You need to check your PC readings on DP. In order to do so, you need to make sure the tank is at least half full, open all the valves on manifolds except outlet valve and open water valve as well (this usually being ignored and could costs you hours try to figure out why it didn’t work). Then, switch on your HPFM system and run HPFM software. Go to “File” – “Calibrate HPFM” – “A/D Setup” and click “Start/Stop”. Increase tank pressure to about 400 kPa and you will be able to see a reading in the window. The DP reading should be somewhere around 0 and PT2 reading should be around 30 mV. Then, close the inlet valve and open outlet valve. The reading should be DP=0 mV and PT2=0.2 mV. If, in both scenarios, the DP reading is + or – 24 out of range, you will need to re-Zero the board. But sometimes, this can be fixed by just adding some new water inside the tank. Then, you will have to bleed the tank for at least 6 hours before operating.
- Check each pressure transducer to see if they are working properly, which mean if they output proper readings to your voltmeter.
- If you have gone through all these steps and still find no luck on running SetZero procedure, please contact Dynamax Technical Support on HPFM for further instruction. Please **DO NOT** disassembles any part of the system.

FAQ: We cannot save the regression values after performing the SetZero option. A "file access denied” box appears when we try to save the regression values.

Answer: This is caused, I assume, by saving your regression value on your calibration CD. If that is the case, please refer to HPFM manual Section 5.1, where ask you to copy your HPFM.CAL and HPFM_CAL_DATA.TXT files from the CD to your Harddrive where you install the HPFM program. If this is not the case, you will need to check if you have specified correct path of your HPFM.CAL file. If not, use option File-NewCalfile to specify the location of your HPFM.CAL file. Then perform Setzero and regression. If you keep experiencing the same problem, please contact Dynamax for further assistance.

5.5 Procedure for finding the Correct Flow Range

Before you start, make sure that you have zeroed the pressure transducers according to **Section 5.4**, "*Setting the Zero Flow Volts*". Setting the zero flow volts must be done at the start of each daily usage of the HPFM.

Make sure that you are correctly connected to the sample in **Section 7**, "*Connecting the HPFM to Roots and Shoots*". To check the flow range, setup for a quasi-state flow:

1. Check the digital pressure gage for the desired flow pressure. Makes sure that the Compressed Air Valve is in the off position (straight up) and the Water Valve is positioned to allow water flow into the filter and subsequently into the valve manifold (8WI and 8WO) system. The valve should point toward the right, parallel to the hose.

2. If you have sampled a root system of the type and species that you will be testing, set the HPFM to the desired flow range by closing off 8WI and 8WO valves on all ranges.

    a. Start the HPFM software and follow the direction of **Subsection 5.7**, "*Quasi-State Flow Measurement*", using the **"Flowmeter"** function from the **"Options"** menu.

    b. Open up all the valves corresponding to the desired range and open the flow out valve. Check for leaks in the lines.

    c. If the software recognizes that the differential pressure between PT1 and PT2 is over 120 kPa, then it will give you a message telling you that the HPFM is out of range. You will need to go the next highest range. The following steps accomplish this:

    d. On the computer running the HPFM software select the **"Pause"** button.

    e. Open up the valves on the manifold for the next highest range. For example, if you are on the **Yellow** range and need to go to the next highest range, which would be the **Gray** range. So you would open the valves corresponding to the **Gray** colored HPLC caps.

    f. Returning to the software click on the **Gray** color range and click the **"Start"** button. You should see a decrease in the differential pressure and the software will stop warning you of an excessively high differential pressure.

3. The working range for the differential pressure is 20 to 120 kPa (3 to 17 PSI). If you attempt to measure dP values above 120 kPa (17 PSI) you will be beyond the calibration range. If you are below 20 kPa (3 PSI) your readings will be more accurate on the next lower flow range.

Note: When you are working with root systems, the differential pressure will normally decrease the longer you have the HPFM coupled to the root. The range should be set accordingly.

CAUTION!

The desired working range for the differential pressure (dP) is 20 to 120 kPa (3 to 17 PSI).

4. If the differential pressure is greater than 120 kPa (17 PSI), then the HPFM software will warn the user. You can then follow Step 5 to increase the range, which will decrease your differential pressure. If the pressure difference falls below 20 kPa (3 PSI), then:

    a. On the computer running the HPFM software select the **"Pause"** button.

    b. Open up the valves on the manifold for the next lowest range. For example, if you are on the **Gray** range and need to go to the next lowest range, which would be the **Yellow** range, open the valves corresponding to the **Yellow** colored HPLC caps.

    c. Returning to the software click on the **Yellow** color range and click the **"Start"** button. You should see an increase in the differential pressure and the software will stop warning you of a too-low differential pressure.

5. Keeping a record of what flow range works with a particular species of a particular size will make it easier to select the correct range on the first attempt.

NOTE: If you fail to pause data collection when changing ranges, you may input a graphical point that causes the graphical display to rescale. Such rescaling is normal, but if you want to remove the bad points just click on the bad points and they will disappear!

5.6 Transient Measurement of Conductance

The transient measurement of conductance of your root or shoot sample allows a quick and fast way to gather the data either in the field or the lab. Quickly acquiring the data is good when dealing with the root systems especially. The root systems as well as the shoots of many plant species have the ability to "repair" themselves and plug off the xylem. Also, there is an osmotic effect in root systems that will increase the osmotic pressure (and therefore reduce the flow) as more water is injected into the root system.

WARNING!

Always proceed with the transient measurement as quickly as safety and sound research practices will allow.

5.6.1 Measurement of the Transient

The **"Transient"** option is used to measure root or shoot conductance dynamically, by measuring flow every few seconds while allowing the applied pressure to change at a constant rate of 5 to 10 kPa s^{-1} = (0.5 to 2 PSI s^{-1}). When measuring low conductance on the lowest flow ranges (**Green** and **Orange**) it is especially important to do prior transient measurements while the HPFM is connected to a solid metal or plastic rod the same diameter as the object you will measure. (See **Section 3** *"Applications and Theory of Operation"* to understand what the data will look like when air bubbles are present. Follow the instructions under **Sub Section 4.5** "*Eliminating air from the HPFM*" to solve the problem).

1. Adjust the needle valve so that the rate of pressurization is at the desired rate approx. between 5 to 10 kPa (0.5 to 2 PSI) per second when the AV is in the pressurize position. Then reduce the pressure to < 7 kPa (1 PSI) by moving AV to the release position. Then open the outlet valve and allow water to flow out of the compression fitting with the **Yellow** range selected. Insert the object to be measured while water is flowing to reduce the chance of trapping an air bubble.

2. Select the desired range and lower the pressure to < 7 kPa (1 PSI). The time interval can also be selected in a drop-down box but the default value of 2s is good for most purposes.

3. Select the "**Transient**" option. And answer the various questions, e.g. providing the filename, title (a brief documentary note up to one line long), the range selected, and the time interval desired, e.g. an integer >1s (2s is usually a good choice). Click on the color range you intend to use in the measurement and enter any comment line you want recorded in the data file. Then click the '**Start**' button. This will bring up a save file dialog box.

Note: that data collection, for the purpose of regressions, will stop after 100 points but the amount of data that can be collected and stored is limited only by the amount of free space on your hard disk.

4. Once you have selected a file name data collection will automatically begin. As soon as the first points appears on the graph start the pressurization. Data are saved as a text file with a 'trn' extension. These files can be examined with the **"FileView"** option or on any text editor. Alternatively, the file can be read into Excel or SigmaPlot.

5. Continue pressurization until PT2 reaches about 500 kPa (72 PSI) or sooner if you detect a problem, e.g., wrong range selected or a leak observed.

6. Stop pressurization by turning the AV to the off position. If you wish, you may continue recording values after the pressurization stops, but it is not recommended when measuring roots since continual flow of water changes the root properties. An example of a typical graphical curve at the end of a transient is shown in Fig. 7. Click the **"Xit"** button to exit to the main menu.

7. If you are measuring a root, quickly release the pressure so as not to change the root by continued perfusion. Often the first transient is poorly shaped because of air bubbles or

leaks or because the rubber in the compression fitting is shifting position. So you will probably want to do one or two more transients on the same object.

CAUTION!

If releasing the pressure quickly causes air to effervesce, this indicates that air in the CAT tank is supersaturated with air. Immediately refill the tank with degassed water to avoid this problem!

X-axis is PT2 in kPa and applies to points plotted as Red **"o"**. The Y-axis is % full-scale. For **"o"** this means % full-scale range for the range tube selected. For **"+"** 100% = 0.5 MPa. F= and P= at the top of the graph are followed by numbers were equal the last value of **"o"** and **"+"** plotted. The X-axis for the **"+"** is time. Each point is separated by the time interval for data collection = 2 s in this case.

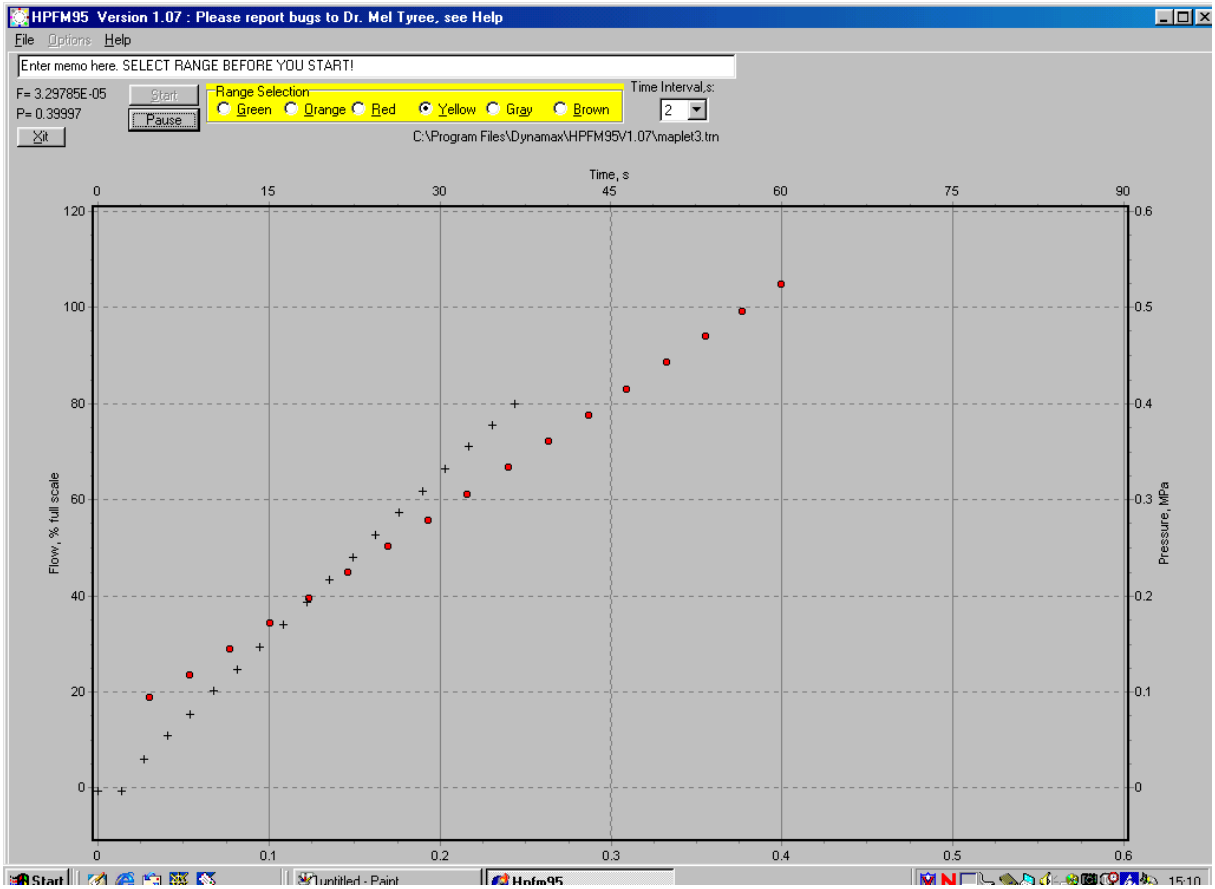


Figure 7
Graphical output of "T" option

The data is saved as an ASCII text under the filename selected. An example of the data file is shown in Table 2. This file can be read into Excel, SigmaPlot or other data analysis software for graphical analysis. But an immediate regression can be done as soon as the transient is finished, by using the **"Regression"** option discussed below.

If you select a filename in step 3, which has already been used, you are given the option of selecting another name or of overwriting the file (which eliminates the data currently in that file!). Alternatively, you can append the new data to the end of the old file (saving the new and the old data in one large file). If you choose to append then you cannot read the file in again for regression analysis because of the limited capability of the regression option. *But you can still do regression on the new data appended to an old file if it is done directly after the **"T"** option is used.* Appended data files still can be loaded into Excel, SigmaPlot or other analysis software. First line is the user-entered title, the second line records the range tube used and the third line records the values and units in the columns below.

Transient into plug with 3 mm OD tubing
Green Slope = 5.69840E-09

Time, s	PT1, mV	PT2, mV	Flow, kg/s	PT2, MPa
0.000	0.00000	0.02413	-1.6415E-08	-0.0014
0.030	0.00000	0.07238	-1.6405E-08	-0.0010
3.000	1.47692	0.21714	4.1943E-08	-0.0000
6.020	3.06520	0.91683	1.0480E-07	0.0049
9.040	4.21978	2.05079	1.5061E-07	0.0128
12.010	5.04029	3.45016	1.8328E-07	0.0226
15.030	5.63810	5.13905	2.0721E-07	0.0344
18.050	6.07179	6.99683	2.2469E-07	0.0474
21.010	6.37656	8.95111	2.3710E-07	0.0611
24.030	6.58755	11.02603	2.4583E-07	0.0756
27.000	6.71062	13.17333	2.5110E-07	0.0906
30.020	6.79853	15.39302	2.5500E-07	0.1061
33.040	6.85714	17.63683	2.5775E-07	0.1218
36.010	6.90403	19.85651	2.6002E-07	0.1373
39.030	6.93919	22.14857	2.6185E-07	0.1533
42.000	6.96264	24.39238	2.6321E-07	0.1690
45.020	6.97436	26.68444	2.6411E-07	0.1850

Table 2
Format of data saved by the "Transient" option

5.6.2 Regression of the Transient

While measurement of the Transient is simply graphing a line of increasing pressure and flow, Regression provides the "best fit" of that graphical line and calculating the slope. That slope is the hydraulic conductance of the measured plant.

1. Select option "Regression". You will have the option to do a regression of the last transient collected in which case you just click on the **"Open"** button. Otherwise, click on the **"Open New"** and select a previous file.

Note: An attempt to load a file with appended data, i.e., two or more transients in one file, will result in termination of the program. So it is a good idea NOT to save data in appended files if you wish to reanalyze them later.

2. After data has been selected, the program will rescale the data so that the maximum flow for P_2 > 150 kPa (22 PSI) is given a value of 100%. A typical re-plot of the data together with a regression line is shown in Figure 8.

Note: that the scale factor, the conductance and the R^2 (=Rsq) for the current regression are displayed. You will have to manually include or exclude points in the regression. Points outside the regression are plotted in red and those inside are plotted in yellow and connected by a regression line. To select the beginning and end points just click on the points. (See Figure 8).

3. To select the beginning and end point just click on the first and last point desired.

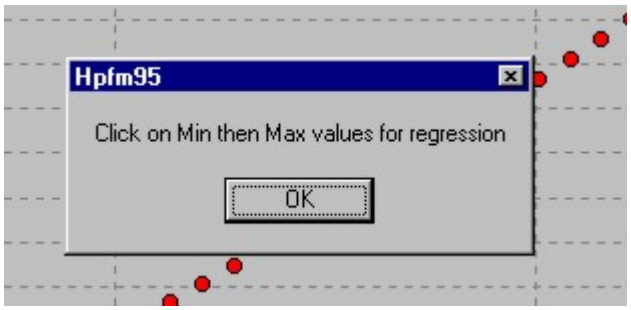


Figure 8
Regressions minimum and maximum window

4. After selecting points press the **"Regress"** button to recalculate and display the new regression values. If there are bad points between the range of yellow points, just click on the bad values (which turn black) and then click on **"Regress"**.

5. The regression values are displayed in the upper left of the display.

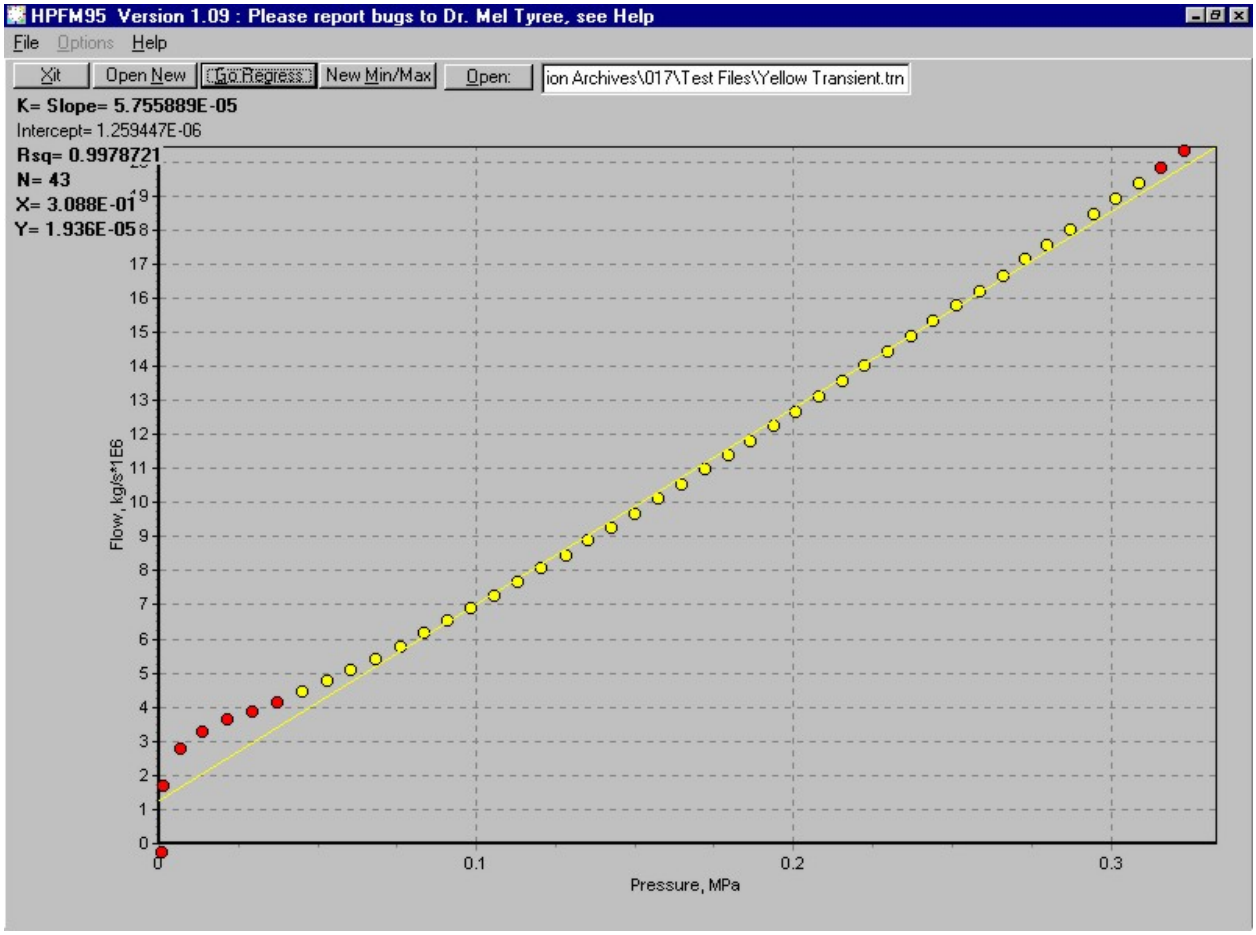


Figure 9
Graphical output of "R" option

Graphical output of **"R"** option done on same data set collected in Figure 8. Y-axis is flow as % full scale. The **"o"** values in Figure 8 have been rescaled so that the maximum value in is now 100% on this graph. X-axis is PT2 pressure in MPa. Values of flow included in the regression are plotted as **"+"** excluded values as **"o"**. The conductance and R^2 values of the current regression are displayed at the top of the graph. Since these is evidence of air in the HPFM (the bump at the lower left of the graph, the user will probably want to redo the regression using values from PT2 = 0.15 to 3.2 MPa.

5.7 QuasiSteady-State Flow Measurement

Quasi-steady state flow meter measurements are usually done on whole shoots, because flow can be approximately constant with constant applied pressure. The resistances are reported rather than conductance, because one of the common aims with quasi-steady state measurements is to measure the resistance of the whole shoot and its components, e.g., leaf blades, petioles, small stems, large stems etc. See **Appendix I** for references regarding Hydraulic Conductance measurements for information on how and why this is done. Follow these steps:

1. Select option "**Flowmeter".**

2. Select the desired flow range. Be sure the valve settings on the HPFM 8-Way valves agree. **Very Important**: Software will give an I/O error and repeat it if you do not set a flow range. The only fix to exit from this error mode is to hit "Enter" very fast and click on "Exit" using mouse. Otherwise, use "Ctrl + Alt + Delete" to stop and restart.

3. Select a time interval. The default value of 8 s is usually good. Values are read every 2 s regardless of the selected time interval. But data are plotted as the mean value for each time interval. Hence, if you pick a time interval of 8 s a point is plotted every 8 s which is the mean of four 2-s readings.

4. Click on the **"Start"** button and you will be asked to select a file name. After the filename is entered the data collection starts. When branches are cut in midday, the leaves are usually dehydrated hence the initial flow rate is determined by the difference between the leaf water potential and the perfusion pressure recorded at PT2. As the leaves re-hydrate, the flow rate of the leaves decreases with time hence the resistance versus time increases. When the leaves are fully hydrated a 'quasi-stable' resistance results as shown in Figure 9.

5. Data collection immediately begins after entering the flow range. The format of the data showing on the screen is shown in Table 3. Values were initially collected every 8s then the collection was changed to every 30s. After about 30 minutes, 'quasi-stable' values of resistance were obtained. Most branches reach a quasi-stable value of resistance in 15 to 45 minutes.

CAUTION!

DO NOT try to change the range in the middle of a "Flowmeter"

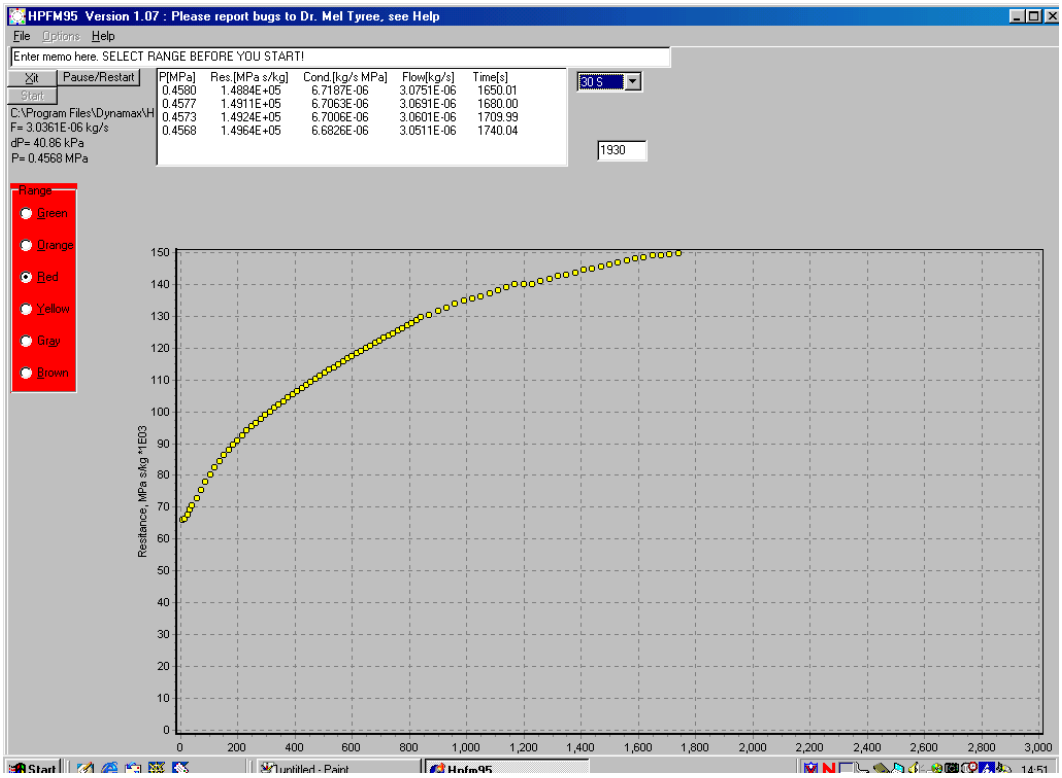


Figure 10
Graphical output of 'Flowmeter' option

6. You will notice that the y-axis automatically rescales as numbers become too large or to small. Sometimes bad numbers are collected that cause an inconveniently large scale change. This usually happens if ranges are changed without pausing or if the compression fittings are tightened without pausing. These bad points can be eliminated by clicking on them and the graph will rescale automatically. DO NOT ATTEMPT TO MANUALLY RESCALE BY DRAGGING THE CURSOR OVER THE GRAPH. ALTHOUGH THIS WORKS TO A POINT IT CAN CAUSE MALFUNCTION OF THE AUTO-SCALING ALGORITHMS.

7. Data displayed graphically above are also saved in text files for future reference as shown below.

Time [s] is the cumulate time; dP[mV] is the raw voltage value of the difference in pressure between PT1 and PT2 (1 mV = 1 PSI = 6.897 kPa), P[mV] is the applied pressure (PT2) in mV and it is shown again in the last column as MPa; the other columns give resistance, conductance and flow respectively. The raw data dP and PT2 in mV are saved in case the wrong range tube was selected. New values for the first 3 columns can be calculated from the raw values of dP and PT2 using the calibration factors in the "View/Set Calibration" Menu.

Quasi-Steady State measurement of 1.5 m Red tube.
Enter memo here. SELECT RANGE BEFORE YOU START!
Red Range = 7.429700E-08 Date/Time 05-Jul-01 14:22:28

Time[s]	dP[mV]	P[mV]	R[MPa s/kg]	K[kg/s MPa]	Flow[kg/s]	P[MPa]
8.02	13.064	63.719	6.6049E+04	1.5140E-05	6.7025E-06	0.43754
16.04	12.859	64.009	6.6096E+04	1.5130E-05	6.5974E-06	0.43953
24.00	12.601	64.105	6.7536E+04	1.4807E-05	6.4652E-06	0.44020
32.02	12.384	64.419	6.9051E+04	1.4482E-05	6.3541E-06	0.44236
40.04	12.132	64.467	7.0554E+04	1.4174E-05	6.2250E-06	0.44269
56.02	11.739	64.829	7.2748E+04	1.3746E-05	6.0237E-06	0.44519
72.00	11.393	65.046	7.5301E+04	1.3280E-05	5.8465E-06	0.44668
88.04	11.095	65.384	7.7814E+04	1.2851E-05	5.6933E-06	0.44901
104.03	10.813	65.577	8.0149E+04	1.2477E-05	5.5491E-06	0.45034
120.01	10.549	65.746	8.2389E+04	1.2138E-05	5.4140E-06	0.45150
135.99	10.338	65.818	8.4352E+04	1.1855E-05	5.3059E-06	0.45200

Table 3
Quasi-Steady State Measurement Numerical Output

'Flowmeter' measurements after 'quasi-stable' values were reached and the subsequent removal of leaf blades is shown in Figure 10. The experiment was done on a maple branch with 10 mm basal diameter and 0.6825 m^2 of leaf area. When the leaf blades are removed over a period of 400s, the flow rate increased and the resistance dropped. When the flow rate exceeded the calibration range, the points turn red. Yellow points indicate values in the calibration range whereas red points are out of range. To bring values back into calibration range, the applied pressure was lowered after the last leaf was removed. The leaves were responsible for > 80% of the whole shoot resistance. When petioles were removed, the calibration range was again exceeded, and the applied pressure was lowered again to bring values back into the calibration range. The petiole resistance was about 5% of the total shoot resistance.

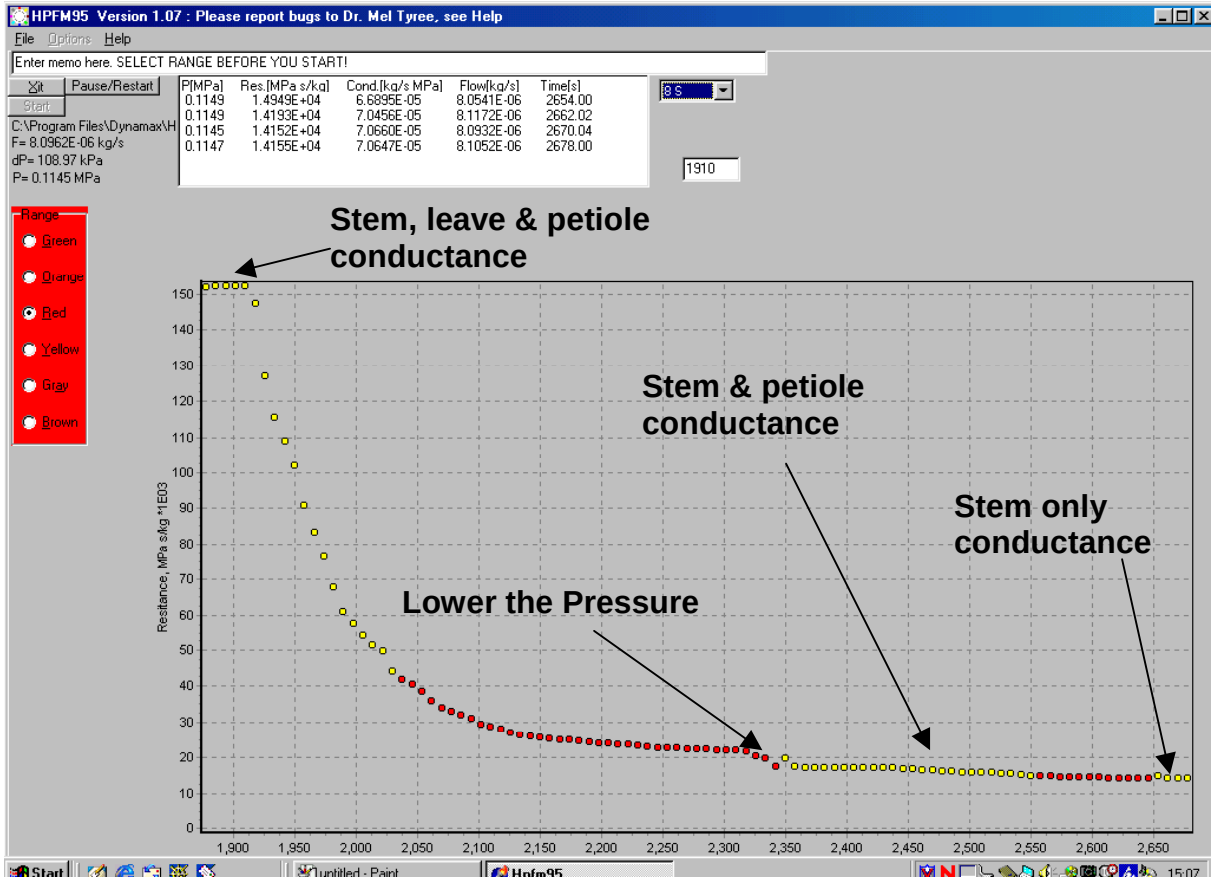


Figure 11
Quasi-Stable Flowmeter Measurements

6. Tips and Cautions for using the HPFM

6.1 Wound Response (plugging of stems)

Frequently, the resistance of whole shoots will rise constantly while doing quasi-steady state measurements. This is generally due to natural wound responses. If the basal 3 to 5 cm is removed, most of the resistance increase is also removed. Many species do not plug at all (See **Appendix II** for a list of species that "plug"). The best way to avoid plugging problems on species with rapid wound response is to do conductance measurements by transient. It is best to re-hydrate the shoot by perfusing at 400 to 500 kPa (58 to 73 PSI) for a few minutes, excise the lower 3 to 5 cm, and then do a transient. HOWEVER, large branches present other problems (see section 6.3).

6.2 Temperature Corrections

The calibration factors used to convert dP to flow are temperature dependent, because K_{CT} is inversely proportional to the viscosity of water and the viscosity of water changes about 2.3% per ^{o}C (Tyree et al 1995). The HPFM will be supplied with calibration curves and the temperature at which the calibration was done. The user of the HPFM should always note the temperature at which measurements were done. The conductance values should be adjusted by the following formula:

$$K_{corrected} = K\ (0.554 + 0.0225\ T)/(0.554 + 0.0225\ T^*)$$

Where:
K = the uncorrected conductance,
T = the temperature at which K was measured, and
T* is the temperature at which the HPFM was calibrated. The resistances should be corrected by the following formula:

$$R_{corrected} = R\ (0.554 + 0.0225\ T^*)/(0.554 + 0.0225\ T)$$

The formulas above correct K- and R-values to the temperature at which the sample was measured. But in addition some people may want to make an additional correction in cases were many different plants were measured at many different temperatures. The temperature correction is approximately 2.4%/^{o}C so if measurements are made over a range of 15 to 35 ^{o}C, stem conductances can change by 48%. Zhang and Tyree have recently measured the temperature dependence of roots and shoots of a number of species summarized below (See Chapter 6.5 in "Xylem Structure and Ascent of Sap – Second Edition" by MT Tyree and MH Zimmermann, Springer-Verlag, 2002).

Species	Root %/°C	SEM	Shoot%/°C	SEM
Acer saccharum	2.74*	0.07	3.27*	0.08
Quercus alba	2.70*	0.03	2.58*	0.06
Picea rubens	2.79*	0.25	2.85*	0.20
Pinus strobus	2.30	0.18	2.44	0.14
Pinus taeda	2.68	0.30	2.50	0.39
Tsuga canadensis	-	-	2.97	0.14
cucumber	2.89*	0.34	0.81*	0.07
squash	2.35	0.05	1.50*	0.18

Data collected by Prof. Zhang in 1998 concerning the temperature dependence of whole shoot and root conductances of seedlings of various plants for plants from 0 to 40 °C. Plots of conductance versus temperature where used to compute mean slope (expressed as percent change) and standard error of the mean of N = 7 or 8 roots and shoots. Means marked with '*' were significantly different from 2.418 (P<0.01), which is the mean value for a narrow capillary tube where the temperate dependence reflects the dependence of viscosity of water only.

Users who want to make temperature corrections should measure the temperature dependence of their study species for the sake of maximum accuracy. Fortunately, in many cases the two temperature corrections cancel out if the following conditions hold: (1) The temperature dependence of the capillary tubes on the HPFM and of the sample are equal. (2) The HPFM and the sample are at the equal temperature, even though the temperatures might differ between measurements. Let us take an example.

Lets assume the HPFM was calibrated at 25 °C and that we want all sample conductances expressed in terms of what it would be at 25 °C. Let us say that a set of measurements were done at 30 °C. The first correction for how temperature effects the calibration factor would require us to raise the sample conductance value by 2.4 x 5 = 12%, which will give us the sample conductance at 30 °C. Then if conditions (1) and (2) are both correct, correcting the sample value back to 25 °C would require us to lower the conductance by 12% again. This has been demonstrated experimentally to be true. Mel Tyree (unpublished) used the HPFM to measure the K of a length of tubing of 0.12 mm ID. When the temperature of the tube ONLY was changed, the value of K_{red} change 2.4%/°C but when BOTH the HPFM and tube temperature changed the value of K_{red} was independent of the temperature and equal to the value K_{red} would be at the calibration temperature.

During lab measurements the HPFM and sample are often at nearly the same temperature, but in the field the HPFM and sample can be at different temperatures, e.g. 5 to 10 °C different, if one is in the sun and the other is in the shade. The best practice is to keep both the HPFM and sample shaded to ensure similar temperatures. Is the sample is wet the evaporation can also lower the temperature below the air temperature.

6.3 Air evenly distributed in the wood of large branches.

Old wood in large branches sometimes contains a large volume of air in embolised wood and wood fiber cells. This causes the quasi-steady state estimate of shoot conductance to differ from the value determined by transient. When no air is present and the leaves are fully infiltrated with water, the values of conductance should agree within 1 or 2% when compared between quasi-steady state and transient methods. The transient values are higher than the quasi-steady state value because part of the water entering during the transient goes to compress the air bubble. But during quasi-steady state measurements, after 40 minutes of perfusion the bubbles are compressed to a 'quasi-equilibrium volume' where further volume change depends on the slow dissolution of the air in water. The dissolution proceeds slowly over many hours in large stems. Oak species contain lots of air in old wood so the transient conductance can be 2 to 4 times the quasi-steady state conductance. The quasi-steady state value is likely to be the most accurate value if stem plugging has not occurred. For more details see: Nardini, A., M.T. Tyree. 1999 Root and shoot hydraulic conductance of seven *Quercus* specie**s. Annals of Forest Science. 56: 371-377**

6.4 Cautions when measuring small flows/conductance or large resistances

The elasticity of the tube connecting the outlet of the HPFM to the object being measured can seriously affect the accuracy of measurements. Small conductance should be measured with small diameter and rigid connection tubes. It is always a good practice to measure flow versus pressure with a solid plug inserted in place of the object to be measured. Conductances are computed from the slope of F versus PT2. Plasticity of connection tubes may give a slight slope of F versus PT2 when measured with a solid plug in place of the object to be measured. Small conductance should be corrected by subtracting the slope observed when doing a transient on a solid plug. The size of this correction is less when small conductances are measured with small diameter, rigid connecting tubes (Figure 12.)

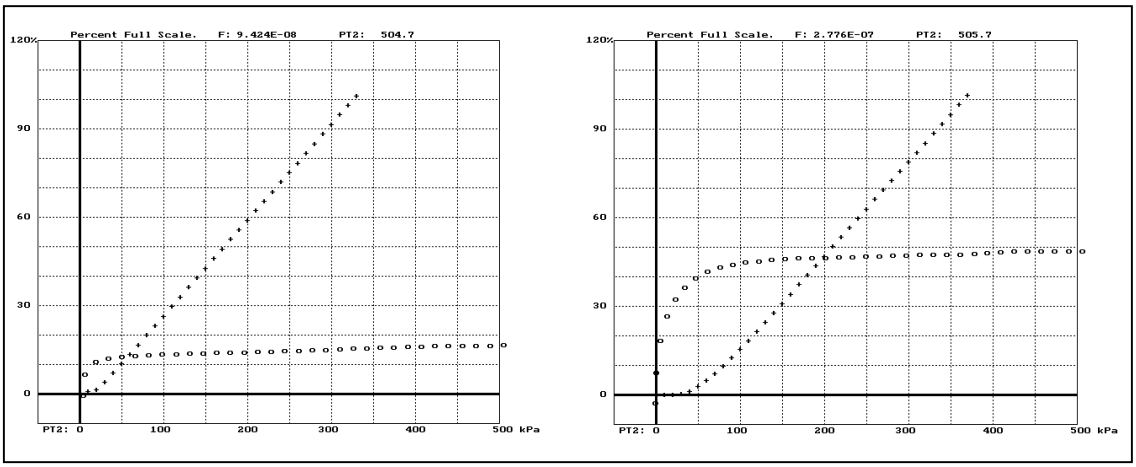


Figure 12
Comparison of transients measured on most sensitive range (Green range)

Flow was into a 1.5 m length of tubing connecting the 8WO to the smallest compression fitting connected to a solid plug 1.5 mm OD. For the graph on the left a 1.5 mm OD plastic tube was used on the right a 3 mm OD plastic tube. The higher plateau on the right is due to the higher elasticity of the larger tube. The slight slope on the plateaus may be due to minor leaks in the 8WO and the compression fittings or it might be due to non-linear elastic effects. Clearly the 1.5 mm tube is preferable for measurements of conductance of an unknown and the slight slope on the plateau should be subtracted from any conductance calculated on an unknown.

6.5 Air Bubbles

Air bubbles in the compression fitting or base of stem will influence the accuracy of conductance measurements, but cannot always be eliminated. Air bubbles are sometimes present in the stems or roots of plants and often cannot be eliminated. Generally, air bubbles can be eliminated from the HPFM. Figure 13 & 14 show some typical transients with the influence of air bubbles.

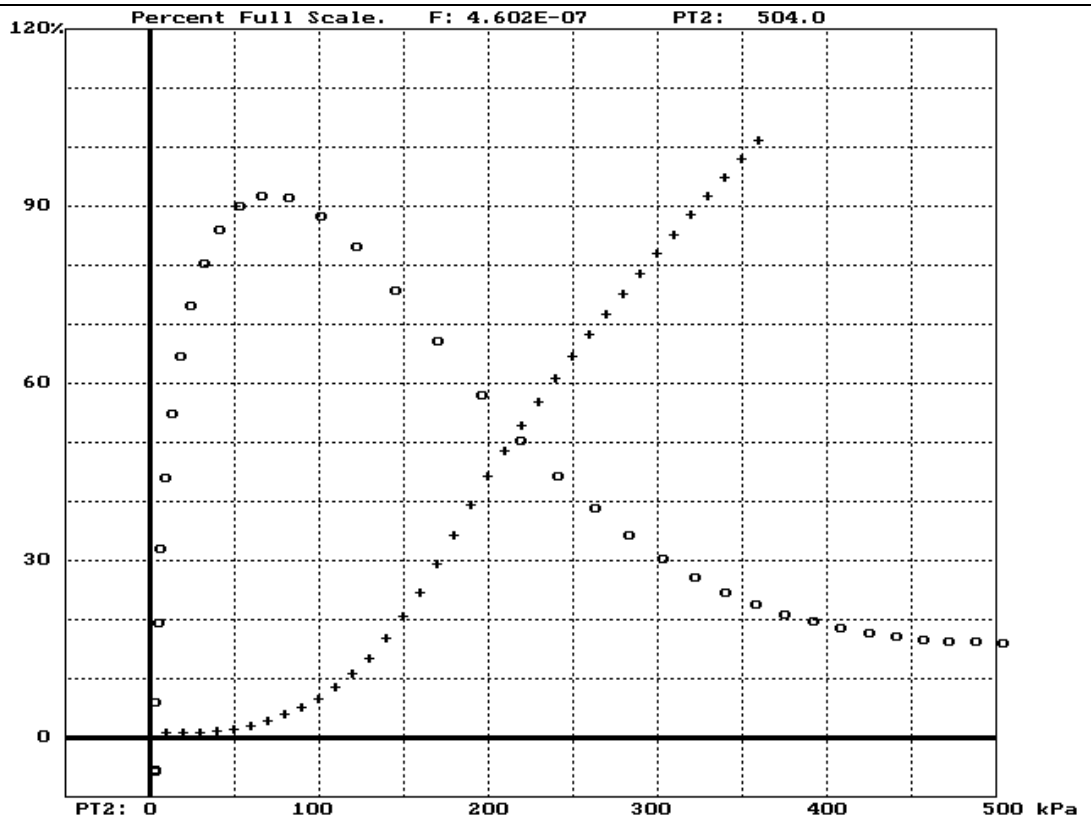


Figure 13
Transient done on the Orange range

Transient into 1.5 m length of red tube made after a very large air bubble was introduced into the 8WO valve. This kind of air bubble can be dissolved slowly by using the methods described in the section "Dissolving air trapped in the 8-way manifold**". NO RELIABLE CONDUCTANCES CAN BE MEASURED WHEN CURVES LOOK LIKE THIS!**

NOTE: A linear transient line (Flow versus pressure) does not mean there is no air in the stems. When air is evenly distributed in the woody shoot, the transient can look quite linear and yet the value of conductance (= the slope) is too big. If the quasi-steady state and transient values of conductance show transient conductance more than 10% than the quasi-steady state value, then you probably have a stem with air more or less evenly distributed throughout the wood.

In Figure 14, this transient has been done on a 1.5 m length of **Red** tube (0.12 mm ID). There is still a small air bubble present but the data are good enough to get a reliable conductance. The transient was collected on the **Orange** range. Figure 13 illustrates what the data look like when collected on the next higher (**Red**) range

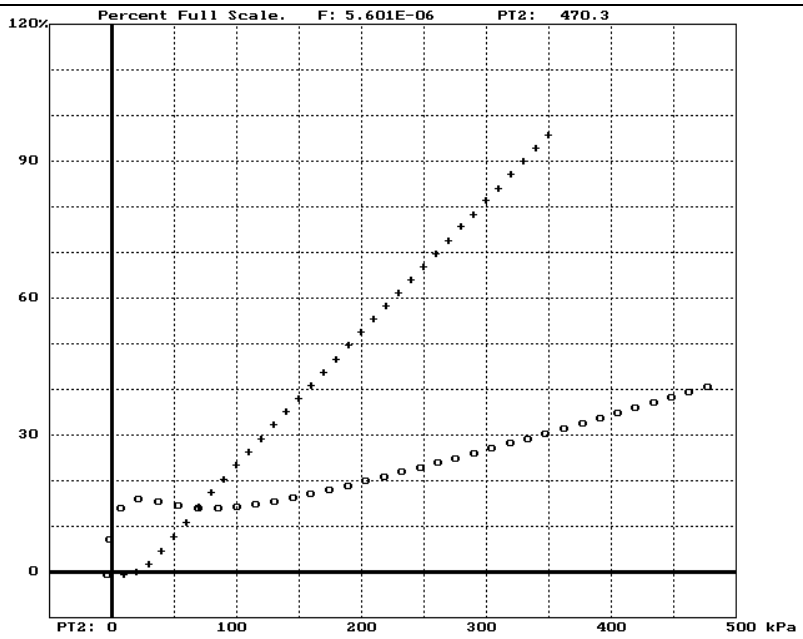


Figure 14
Transient collected on the Orange range

Figure 15 is a repeat of the transient done above with a small air bubble in the 8W0 manifold. The transient was re-measured on the **Red** range. The slope of this curve should still give a valid conductance although the bubble can be eliminated completely.

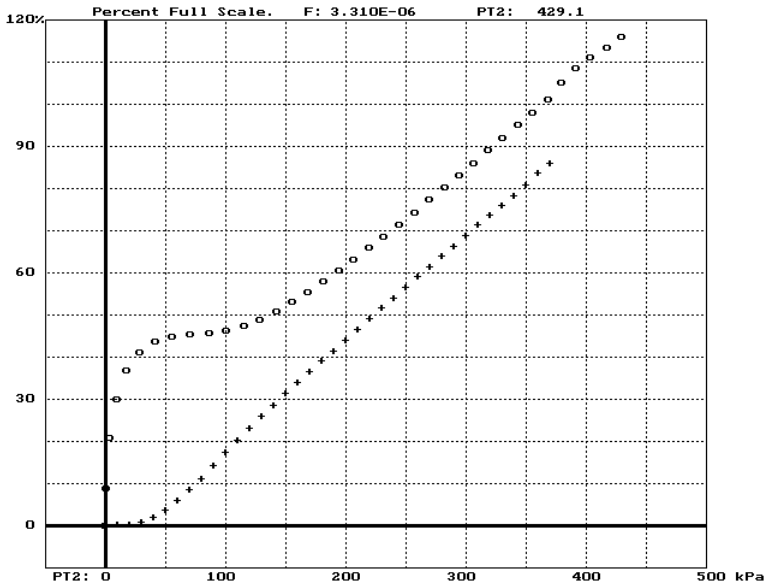


Figure 15
Transient re-measured on the Red range

6.6 Safety

When working with tools, one should always consider safety. Razor blades can cut more than the xylem. So can saw blades. Being careful cannot protect you all the time. Wear gloves when using sharp objects. Make sure that you are cutting away from your body (and fingers) as well as away from other people. Wear safety glasses when using a saw blade. If you are in the field, and you are excising trees (or small parts of large trees), make sure that you wear the proper equipment, i.e., hard hats, steel-toed boots, when cutting. Other body protection may include sunscreen, long sleeved shirts full-length pants, and hearing protection.

Also, consider the safety of those around you. Be aware of what you are doing and how it will affect the area around you and the people in that area. Are they wearing the proper safety attire? If you are in a lab, are they wearing proper protection as well?

7. Connecting the HPFM to Roots and Shoots

Connecting the HPFM to the Root or Shoot of choice is important to obtaining a quality reading. If the connection leaks or if there is air in the connecting apparatus, then the HPFM will show a higher than normal flow or show an abnormal transient due to compression of the air.

7.1 Root and Shoots

The HPFM, with practice, can be connected to the root, gather data, removed from the root and readied for travel in about 10 minutes. The HPFM can probably be connected to a shoot in less time than a root.

The steps for setting up a root or shoot are:

Photo 8
Cutting a Stem

1. Measure the diameter of the sample where you plan to excise or cut the sample plant or tree. Make sure that there are no anomalies such as grafting scars, limbs, old limb scars, or excessive damage that might cause problems with the compression coupling creating a sealed system. Keep in mind that the largest object that the HPFM can be connected to is 50mm with the included compression couplings. Measure the object that you wish to sample and make sure that you are in range before excising the shoot or trunk! The larger sampled sizes, 40-50mm, will probably require the bark to be removed. Any rough surface that will affect the seal of the compression fitting will need to be removed or smoothed with sandpaper.

2. Excise the shoot or cut down the trunk. Points to remember before and during the excise procedure are:

   a. Rough bark will need to be removed down to the xylem. This can be considered when measuring the sample. Even if the bark is smooth, water flow could move between the xylem and the bark, or between the bark and the compression coupling, the bark should be removed.

WARNING!

Do Not Remove the Xylem from the sample.

   b. When cutting a root sample, never cut the sample too low to the ground. If you are cutting a shoot sample, do not cut too close to a major stem. Give yourself plenty of room. Use the rubber compression seal from the compression coupling you plan to use to check the distance. The point to excise or cut should be:

i. For sizes up to 20mm, leave 2-5mm of root stem above the top of the rubber compression seal.
ii. For sizes from 20mm to 50mm, leave 5-10mm of root stem above the top of the rubber compression seal.

Note: Check the distance below the rubber compression seal. Can you see leaks? Can you perform the installation of the coupling easily?

c. Cut or excise with a smooth level cut. The cut should always be perpendicular to the stem.

d. Use a razor blade (included with the HPFM) to remove the bark and related material to the depth of the cambium.

Photo 9
Removing bark from the stem

3. After having cut and prepared the stem, slip the nut and aluminum compression ring over the root stem. Make sure that the washer is as close to the size of the stem as possible. The aluminum compression ring should still be easy to slip on or off.

4. Place the rubber seal over the prepared area of the root stem. The seal should be snug, but should not have to be forced. If the compression seal is too small, use the next largest seal. If the rubber seal is too big, use a smaller one. The rubber seal should be applied with out a lubricant onto the sample and should have some light to moderate friction when twisted. Approx. 1cm of stem should be protruding from above rubber seal as shown in Photo 10. If not, go back to step 2 and remove more bark.

Photo 10
Installing the Compression ring

WARNING!

BE CAREFUL with Knives, Razor Blades, Saws and other TOOLS.

5. Apply a light coating of the included G4 silicone grease to the threads of the head of the coupling as well as to the inside cone. The area inside the cone that requires the lubricant is where the compression seal will compress into the head by the nut. Again, a light coating is all that is required.

Photo 11
Greasing the Coupling

CAUTION!

Make sure to apply a light coating of G4 Silicone grease to the outside surface of the rubber seal. This will prevent binding and eventual destruction of the seal and coupling surface.

6. Compress the rubber compression seal into place by holding the coned head in place above the compression seal while tightening the nut into it. Do not move the head as the unit is being tightened, only the nut. Compress the nut until it is firm and there should be no fluid losses. Note that the root stem should stick out slightly (approx. 1cm) above the rubber compression seal. If it does not, remove the cone and compression fitting and remove more bark as per step 2. If the rubber compression seal closes around the end of the stem, then an inaccurate reading may occur.

Photo 12
Tightening the Coupling

7. Remove the HPLC compression-fitting cap (the colored tubing fitting at the top of the compression coupling). Be careful. There should be at least 2 small viton “O” rings inside the connection.

8. Take the Hypodermic syringe and fill the compression unit with de-gassed water. This water could come from the water carried in the Refill Kit apparatus.

WARNING!

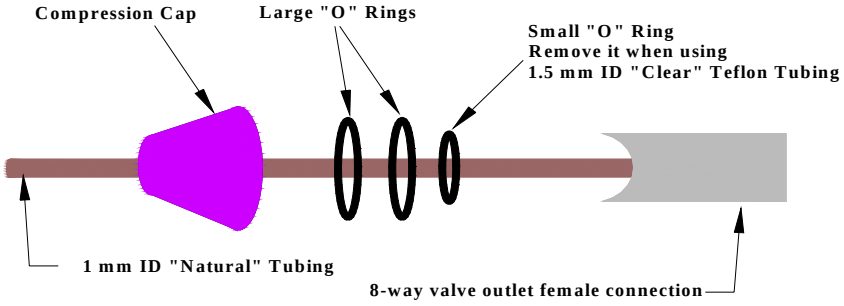
The HPFM comes with a hypodermic syringe that has a 1 3/8" blunt point "needle".
For safety only use this type of needle.

Stick the needle down into the HPLC tubing connection of the cone and depress the hypodermic syringe filling the open area inside, displacing the air. As the air is replaced, use the needle to help remove air bubbles or pockets, using the velocity of the water moving out of the hypodermic needle. Once you have filled the open area of the compression fitting with water and have removed all the air, pull the hypodermic needle out while still depressing water out to fill the displacement of the needle itself.

Make sure that you have water on top of the open area of the HPLC connection with no air pockets below.

Photo 13
Removing Air from the coupling

9. Note the tubing you are using. If you are using the smaller 1mm ID "Natural" HPLC tubing recommended for use with small compression coupling (1-20mm), then you will need two large viton "O" rings with one small viton "O" ring for connecting the tubing to the compression coupling. If you are using the larger 1.5mm Teflon clear tubing, you will only need the two larger "O" rings for connecting with larger couplings (19-50mm).


Compression Cap
Large "O" Rings
Small "O" Ring
Remove it when using
1.5 mm ID "Clear" Teflon Tubing
1 mm ID "Natural" Tubing
8-way valve outlet female connection

"O" Ring Layout Diagram

CAUTION!

Make sure that you do not have air in the compression-coupling unit, the tubing leading to the compression unit, or in the HPFM.

The steps for connecting the tubing are:

a. Carefully remove the "O" rings from the HPLC compression-fitting cap.

b. To prevent FEP Teflon tubing from slipping, take the included razor blade and carefully rough up the end of the tubing where the viton "O" rings compress into the tubing. Slanted small scrapes seem to work best to hold. The more problematic slippage may require that you use a razor blade. Remember to keep the razor from slicing into the tubing or a finger.

c. Place the HPLC compression-fitting cap on the tubing that you are using for your sampling.

Photo 14
Connecting the "Clear" tubing w/ 2 larger "O" rings

d. Make sure that the tubing is not constricted by previous compression in the fitting. If it is, use a razor blade to cut off a small part of the tubing.

e. Place the necessary "O" rings on the tubing. If you are using the smaller tubing and require 3 "O" rings, place the smaller "O" ring on the tubing first, followed by the two larger "O" rings.

f. Make sure that the pressure is >5 PSI (35 kPa). Open the valves on the HPFM, allowing the tubing to bleed the water at a slow drip as shown in photo 14 above. This will remove air from the tubing.

g. Place the tubing into the compression fitting on the top of the compression coupling and move the HPLC compression fitting cap down. Make sure that the tubing stays solidly inside the acrylic cone of the compression coupling.

h. Make sure that you have water flowing and begin to screw the HPLC compression-fitting cap into the compression fitting on top of the compression coupling. Make sure that it is snug, but do not over tighten. Two or three turns is all that is usually required after feeling the resistance of the" rings.

NOTE: Excessive squeezing of the tubing by the compression fitting and "O" rings may restrict flow on the larger volume ranges of the HPFM.

CAUTION!

Do not over tighten. Never use tools to tighten. You can compress the connection tubing, stopping the flow of water and give you erroneous results.

i. Water should have accumulated in the cracks and crevasses of HPLC compression fitting. Turn off the water flow by closing the Flow Out valve on the 8WO manifold. Turn the fitting cap until it is tight. Remember the warning above in step 9f.

j. Give the tubing a slight pull to see if the tubing will stay in place. If it does, great go on to step 10. If not go to step 9b.

10. Open the flow out valve, making sure that there are no leaks. If there are leaks, repeat steps 7 through 9. If there are none, close the flow out valve and you are ready.

NOTE: A common problem with connecting large stems to compression fittings is that the fitting slips off during pressurization. One way to prevent this is to obtain a few small wood screws and screw 2 to 6 of them between the end of the stem and the upper surface of the rubber stopper. Leave the heads of the screws a few mm above the wood. This will prevent the stopper from slipping past the screws. The effect of the screws on the root or shoot conductance is very small! On smaller stems, too small for screws, try to make the cut at a nodal swelling and mount the stopper just below the swelling.

8. Disconnecting the HPFM

8.1 Valves Settings for moving the HPFM

The valve setting for moving a HPFM is simply in normally to leave the CAT tank pressurized and closing the outlet valve on the 8WO. The reason this deserves a section is that the valve position is important.

The minimum required work, if these valves are not closed, is that the 8WI and 8WO manifold system will need to be bleed again. The maximum would be bleeding off all water and purging the air bubbles from the entire system!

8.2 Disconnecting and moving the HPFM

Specifically designed to be portable, the HPFM has several options that enhance this characteristic. One option is wheels and the other is a backpack frame to carry the HPFM into areas usually not accessible for research.

The HPFM, before moving, must be prepared. Preparation will keep the HPFM ready for the next sampling, keeping air pockets or bubbles from entering into the HPFM system. The steps for preparing the HPFM are:

a. Close off the purple outlet valve on the 8WO and leave the CAT tank pressurized.

b. Disconnect the Compression Coupling from the connecting tubing by loosening the HPLC cap on the coupling.

c. Disconnect the tubing carefully holding the tubing above the height of the HPFM. This will minimize gravity loss of the water in the tubing and the connected HPFM system. One suggestion is to use a rubber band to hold the tubing to the frame.

d. Remove the Compression Coupling from the sample by loosening the Compression Nut from the Coupling. Slip the acrylic housing off the Compression Seal.

e. Slide the Compression Seal off the sample by twisting lightly as you pull the Seal off.

f. Place the seal back into the Compression Coupling for storage.

9. Maintaining the HPFM

9.1 Changing the Water Filter

The HPFM water filter, under normal conditions, should be replaced annually. Under heavy loads, the filter should be replaced at least twice a year. To change the water filter follow these steps:

1. Release the compressed gas pressure making sure that the Captive Air Tank pressure is 0 PSI (0 kPa). This is accomplished by turning the Compressed Air Valve to the right, releasing the pressure slowly (1-1.5 PSI per second, 6.9-10 kPa per second).

2. Loosen the steel strap that holds the water filter to the frame. It is not necessary to remove the steel strap. You should loosen the strap until you feel that the filter can slide up or down.

3. Loosen the steel straps that hold the tubing to both ends of the water filter. Loosen the top strap first. Please be prepared for water to leak. Cover the Digital Pressure gage with a small plastic bag in order to keep water from splashing onto the electronics of the gage. Use rubber bands or tie wraps to hold the plastic bag in place.

WARNING!

Always cover the Digital Pressure Gage when working on the mechanics of the HPFM. Water can leak or splash onto the electronics of the gage making the gage inoperable.

4. Pull the tubing off the top connection of the water filter. Use a slight twisting action while pulling backward. Hold the water filter firmly. After removing the connection, hold the tubing up to minimize the spillage of the degassed water. Also make sure that the steel strap is in place for the new water filter.

5. Slide the filter down, removing the water filter from the strap that is binding it to the frame.

6. Hold the filter and with a slight twisting action remove the tubing from the bottom of the water filter. Make sure that the steel strap is in place for the new water filter.

7. Check the flow direction arrows on the new water filter. Make sure that the water filter is installed in the right direction.

8. Take the new water filter and with a slightly twisting action push the filter on to the bottom tubing.

9. Slide the water filter into place. Tighten the strap that holds the water filter in place. Make sure that the filter ports (Red plugs on the top and bottom of the water filter) are

facing toward the back of the HPFM. Also make sure that the water filter is in the same location as the old filter. This will make it easier to handle the tubing connections.

10. Using a slight twisting action push the tubing on to the top filter connection.

11. Tighten the two steel straps that hold the tubing in place on the water filter.

12. Follow the instructions in **Section 4.5**, "*Eliminating Air from the HPFM*".

13. Discard the old water filter properly, draining all the water from the old filter.

9.2 Checking the Battery

1. Check the power light to see if it illuminates. If not, try charging the HPFM battery for a few hours.

2. If you have tried to charge the battery and the power light will not illuminate, check to make sure that you have power to the charger. In the U.S. and other places this would be 120VAC 60Hz. In Japan, the power to the charger would be 110 VAC 50Hz, and in Europe the power would be 220 VAC 50Hz. Check the power charger to see which one you have. Make sure that you are connected to the correct power source with the correct charger.

3. Remove the top panel from the HPFM. Note placement of the foam cushion and remove it. Check the battery terminals to make sure that the connections are secure.

4. Using a multi-meter check the voltages on the battery. A fully charged battery should show at least 12.5 volts. If it does not, replace the battery according to **Subsection 9.3**, "*Changing the HPFM Battery*".

5. If the battery is not charged fully, then connect the charger and measure the voltage with the HPFM off. The voltage should be 12 VDC or higher. If it is not, check the charger power source. If it is good, replace the charger.

6. Leave the charger connected for 12 hours. Disconnect the charger from the HPFM. Check the voltage with a Multi-meter. If it is less than 10 VDC, then replace the battery according to **Subsection 9.3**, "*Changing the HPFM Battery*".

8. If you are still having problems with the battery, please contact your local distributor or Dynamax for repair.

9.3 Changing the HPFM Battery

The HPFM has a compartment located at top of the unit toward the back. This compartment contains the electronics for the HPFM and the battery. This battery is a 12VDC 4.5Amp Hour Gel-Cell type and may require replacement every three to four years depending the use of the HPFM. To replace the battery:

1. Loosen and remove the 4 slotted screws that hold the top panel in place. Put the screws in a safe place.

2. You should be able to see the battery mounted on its side. Make a note of the placement of the foam cushion. Remove the foam from around the battery.

3. Push the battery back and remove the metal extender that holds the battery securely against the back of the compartment. The extender is threaded into the compartment side.

4. Disconnect the battery from the circuit. There are two connections, the positive terminal (red) and the negative terminal (black). Remove the positive terminal first.

5. Remove the old battery from the compartment and replace it with the new battery. Make a note of the position of the old battery for positioning of the new battery.

6. Replace the threaded extender that holds the battery in place. Make sure that you push the battery firmly while threading the extender so that the extender will thread straight.

7. Replace the Black wire to the negative terminal. Replace the Red wire to the positive terminal. Make sure that the wires are in place securely.

8. Replace the foam cushion according to its former placement in the compartment.

9. If the battery is charged, turn the power on to make sure that the unit is in place and working.

10. If the battery is not charged, charge the battery overnight and check the power light before replacing the top panel.

11. If the unit does not work, remove and check the connections to the terminals of the battery. Check the voltage to the terminals of the battery with a multi-meter. The voltage of a fully charged battery should be 13.5 to 13.9 Volts Direct Current.

12. Once you are sure the battery is working properly, replace the top panel with the screws removed previously.

9.4 Changing the Digital Pressure Gage Battery

A 9V battery powers the digital pressure gage. If the operating procedures are followed correctly and the digital pressure gage is turned off when the system is not in use this battery is sufficient to last several years. However, if the display becomes dull and numbers are incomplete and difficult to read you will need to replace the battery. Follow the steps below:

1. Turn the gage off
2. Unscrew the back panel of the gage and unclip the 9 V battery.
3. Insert the new 9V battery being sure to clip the contacts on securely.

9.5 Zeroing the Digital Pressure Gage

The digital pressure gage used on the HPFM is of high quality with an accuracy of less than 5%. Reading drift is uncommon but can occur overtime. If you consider that the gage is not reading the applied pressure accurately begin by checking it against the Pressure recorded by PT2. (see **Section 10.3** "*Manual Test of the Pressure Transducers*")

1. Open all the range valves on both the Inlet and Outlet manifolds except the **Purple** outlet valve. This must remain closed to allow the pressure being applied to the system to be read on PT2.
2. Apply a pressure of 100kPa (14.5 PSI) and read the corresponding millivolt (mV) value on the AtoD system panel. Remember that the Transducers are calibrated so that 1mV = 1PSI
3. The reading in millivolts should be between 14.2 and 14.8 or 2% of the equivalent 14.5 PSI (100kPa) displayed on the Gage.
4. Exhaust the air and read the pressure on PT2 once again. Both PT2 and the gage should read zero.
5. If the gage has failed these checks take a small flat screwdriver and insert it into the zero set slot on the back of the gage. Very gently turn the potentiometer until the gage reads zero.
6. Repeat step 3, 4 and 5 to check that the gage is now set correctly.

10. Trouble Shooting

10.1 PC-HPFM Communication

Please note that PC software automatically detects the HPFM. If the HPFM is not automatically detected the following an error message is displayed. If you receive this error message attempt the following:

Figure 16
HPFM Error Message

1. Make sure that power to the HPFM is turned on.

2. If the power is switched on but the power light is not illuminated check that the battery is connected and charged. (see **Section 9.2** "*Checking the Battery*")

3. Make sure that the parallel communications cable is directly connected to the computer and the HPFM. Do not run the cable through any third part devices such as Zip Drives and external CD-Writers.

4. Confirm that the HPFM communications cable is connected to a printer port: LPT1, LPT2 or LPT3. It should usually be LPT1.

5. Change the parallel communications cable between the HPFM and the PC to rule out a bad cable.

6. Confirm there is not a communications conflict with a printer driver. Uninstall all printer drivers from the computer connected to the HPFM.

7. If all of the above suggestions have not overcome the communications problem it may be that the A/D board on the HPFM is defective.

Note: This last possibility will require service by a qualified electronics technician. Please contact Dynamax Inc. or your Local distributor and be sure to describe your problem in detail citing all the steps you have performed previous to this point in attempting to trouble shoot your problem and any use of the system since it last worked. For example, *"We left it at our remote experimental site and it rained for a week", "I dropped it from a 50ft tower in a forest canopy study!"*

10.2 Computer Reading Problems

If you have trouble with the readings on the computer, attempt the following procedure:

1. Connect the small **Red** HPLC tubing to the Flow Outlet of the HPFM.
2. Make sure that you have pressurized the Captive Air Tank (CAT) to at least 275 kPa (40 PSI).
3. Set the computer software program to read a quasi-state steady flow rate.
4. Set the flow range for a differential pressure of less than 18 mV (1mV = 1 PSI)
5. Read the differential pressure and add this to the PT2 pressure. This added pressure should be close (within 2 PSI) to the pressure at the digital pressure gage.
6. If the sum of the differential pressure and the pressure at PT2 is not close to the digital pressure gage, then the small pressure transducers (PT1 and PT2) may need to be zeroed. Zero the transducers per the instructions in **Section 5.4**, "*Setting the Zero Flow Volts*".

10.3 Manual Test of the Pressure Transducers

1. From the File menu choose **"Calibrate HPFM"** then; click the **"A/D Setup"** button.
2. Click on the **"Start/Stop"** button and the values of dP and PT2 will be read and updated twice a second and will appear in the text boxes **"Edit10"** and **"Edit11"**respectively. Text boxes **"Edit12"** and **"Edit13"** give the binary values from the A/D board for dP and PT2 respectively.
3. If the pressure applied to a transducer exceeds the maximum range of the transducer will display 18 mV and show Out of Range

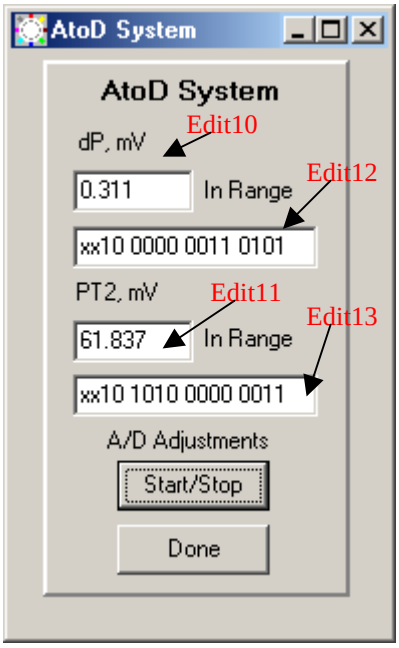


Figure 17
A/D Setup Screen

4. In “A/D Setup”, if the value is “out of range”, perform the following check:

   a. Make sure the system is bled clean of air bubbles.

   b. Increase pressure to 500 kPa (73 PSI), open all the valves except Inlet, so PT1 = PT2 = 0 kPa (Low Pressure). The values appear in the text boxes should match PT2 ≅ 0 mV and dP < 0.1 mV. See Figure 17-1 “Low Pressure Test”

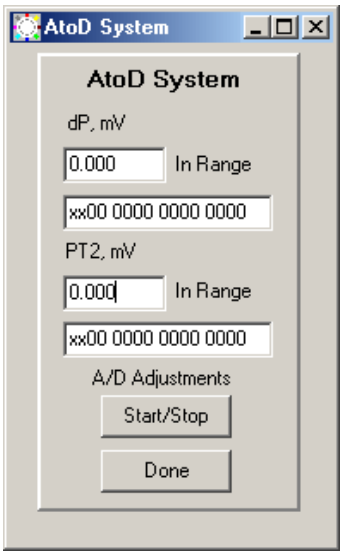


Figure 17-1
Low Pressure Test

   c. Close Outlet and open Inlet valve, so PT1 = PT2 = 500 kPa (73 PSI High Pressure). PT2 should change to about 72 mV, which is 1 mV per 6.895 kPa (1 PSI), and dP should remain at < 0.1 mV. See Figure 17-2 “High Pressure Test”

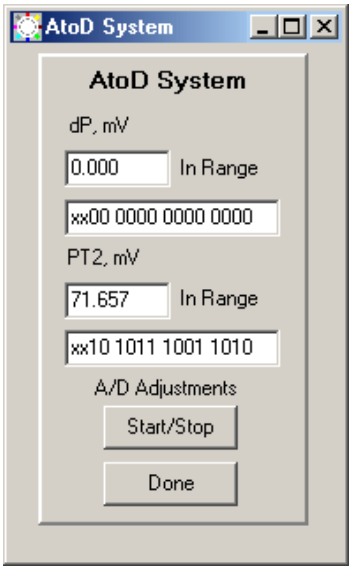


Figure 17-2
High Pressure Test

11. Calibration of the HPFM

Normally, the HPFM will not require frequent calibration. Dynamax recommends that calibrations only be performed by our fully trained technicians at the factory, or by trained and fully qualified Dynamax distributors. Inexperienced users or people without technical electronics training should not attempt calibrations.

For qualified technicians to calibrate the HPFM, a high precision electronic balance, with a 4-decimal place resolution is required. The balance must be able to weigh at least 200 g to the nearest 0.0001 g and have a serial interface for communication with a PC. The HPFM95 software supports RS232 communication with Mettler and Satorius balances set for 8 bits and 2400 baud. The Mettler balance can be used with or without parity check.

Select a container (A) that can hold up to between 150 and 200 mls of water and place it on the balance with a water-filled tube connecting the HPFM to the balance as diagrammed in figure 17 below. Connect an RS232 serial cable between the balance and your computer and the parallel printer cable between the HPFM and the computer.

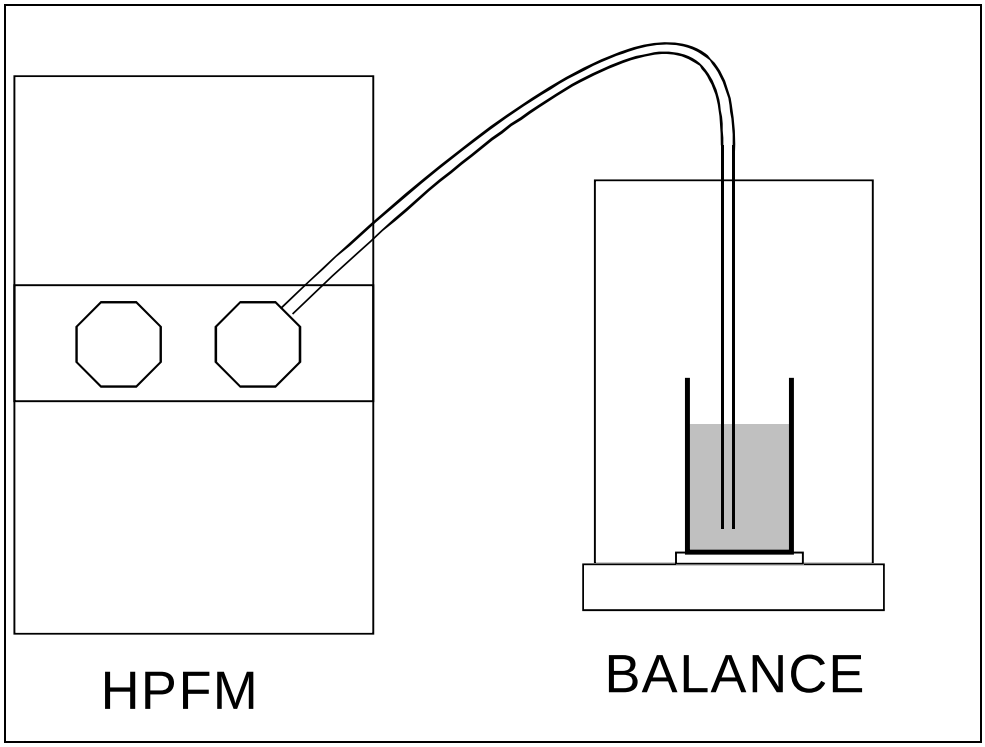


Figure 18
A container on the balance with a water-filled tube connecting the HPFM to the balance

Before you start calibration, you will have to measure the displacement factor of the container with tube. The tube should not be touching the sides of the container nor should it be resting on the bottom. As water flows into the container, the balance will register a slightly larger weight gain than the actual mass of water added to the container because of the volume of water displaced by the tube. Here is how you measure the displacement factor. Find another container (B) and fill it with about 100 mls of water.

1. Weigh container B with water on another balance and note the weight.
2. Note the current weight of container A on the balance.
3. Pour in all the water from container B into container A and note the new weight.
4. Weigh the container B again.

An example of the weights you might measure and the calculation of displacement factor is:

Wt. Container B before:	154.3562 g
Wt. Container B after:	58.3440 g
Wt. of water added to A:	96.0122 g
Wt. Container A after:	175.0022 g
Wt. Container B before:	74.9900 g
Wt. change of balance:	100.0122 g

The displacement factor is 96.0122/100.0122 = 0.96000. The computer, to calculate the actual mass of water entering the balance during calibration, uses this displacement factor. The computer reads the balance at user-defined time intervals. Lets say that in 64 s the weight of the balance changes by 1.0000 g. HPFM95.exe multiplies this number by 0.9600 to get the actual mass of water that flowed into the balance (0.9600 g or 9.6 X 10^{-4} kg and divides this by 64 s to get the volume flow rate = 1.5 X 10^{-5} kg s^{-1}). This displacement factor needs to be edited into the top line of an ASCII file supplied with the software. HPFM95.exe reads this value when it starts doing a calibration. The filename containing the value is HPFM.dat. This file can be edited using 'FILE' '**FileView**'. Edit the text then save it using the '**SaveFileView**' option. Alternatively, use NotePad or WordPad to perform the same operation. Word processing packages, e.g. Microsoft Word or Word Perfect will save the data as a NON-ASCII unless you know what you are doing and then HPFM.exe will not be able to read it!

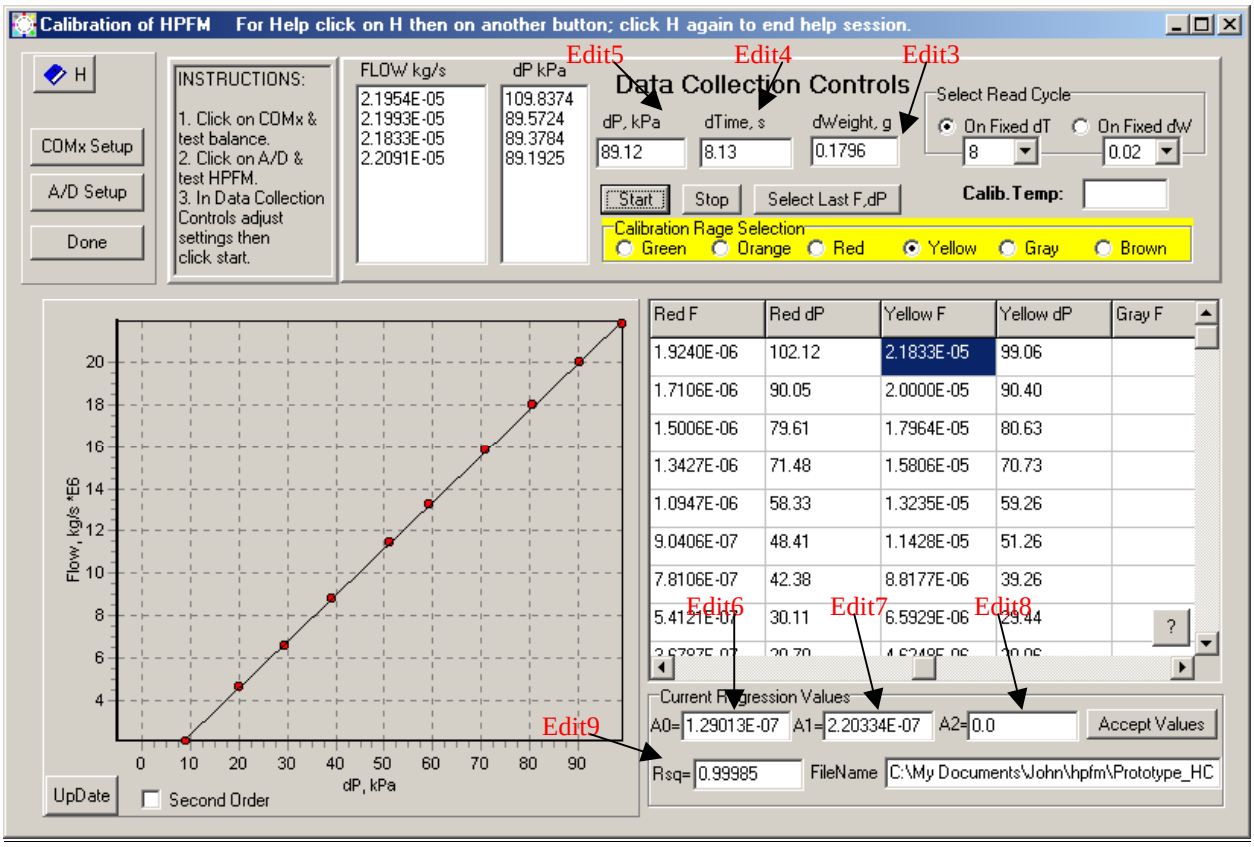
CAUTION!

If at this stage you are unsure about what you are doing then be sure to save a backup file of HPFM.CAL before you measure and enter new calibration factors.

11.1 Calibration Window

To reach the calibration window start HPFM95 by clicking the HPFM95 Icon then, click on '**File**' menu and select '**Calibrate HPFM**'.

Figure 19
Calibration window



The window contains; (1) A 'Data Collection Controls' block, (2) A graphical block, and (3) a data table. In order to do a calibration; your computer must be connected to the HPFM and to a digital balance. Connection to the digital balance must be via a COM port and you must have the manual to your balance to read information on COM port communication protocols. You also need a thermometer which is used to read the room temperature which is entered into the text block for **"Calib. Temp:"**

11.2 Calibration of Pressure Transducers: PT1 and PT2

NOTE

This procedure is recommended only for the factory or certified technician.
It is required ONLY if PT1 and PT2 are changed.

The pressure transducers can be calibrated without a digital balance, but you will need to use a voltmeter to read the output voltages from the HPFM transducers (PX136). The voltmeter has to have in input impedance of at least 10 MOhms and should be capable of reading to the nearest 0.01 mV. The PX136 transducers have a nominal output of 1 mV = (1 PSI) (accurate to 1%) 6.89 kPa and a zero offset at zero PSI that is usually less than 0.2 mV.

Click on the **"A/D Setup"** button. A calibration panel (shown to the left) will appear. When you click on the **"Start/Stop"** button the values of dP and PT2 will be read and updated twice a second and will appear in the text boxes **"Edit10"** and **"Edit11"** respectively. Text boxes **"Edit12"** and **"Edit13"** give the binary values from the A/D chip for dP and PT2, respectively. Calibration involves three phases: (1) Calibration of PT2, (2) Balancing of PT1 and PT2, (3) Calibration of dP.

11.3 Calibration of PT2

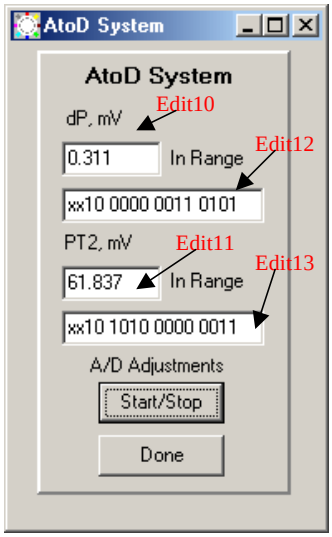


Figure 20
A Calibration Panel

Close the inlet valve on the HPFM and pressurize the captive air tank to approximately (560 kPa or 80 PSI). Select the **Red** range. Open the outlet valve and momentarily open the inlet valve until you see water coming out the outlet tube then close the inlet valve. Place the end of the outlet tube within 1 cm of the height of the PT2 transducer. Do the following steps:

1. PT2 should now read zero mV. If it does not, then adjust the PT2 Zero pot on the A/D board until the value equals zero.

2. Next, close the outlet valve and open the inlet valve. Measure the output of PT2 using the voltmeter. The best place to read the voltage is on the input pins of the amplifiers on the A/D board. Adjust the PT2 gain pot until the value in **"Edit11"** equals the value read by the voltmeter.

3. Close the inlet valve and open the outlet valve and check that PT2 still reads zero. If it does not, then readjust the PT2 zero pot and then repeat steps (2) and (3).

11.4 Balancing PT1 and PT2

Make sure the captive air tank is still at about 550 kPa (80 PSI). The objective in the following steps is to adjust the PT1 gain pot so that PT1 = PT2 at all pressure values; since dP = PT1-PT2 we want dP = 0 at all pressures. Repeat the following steps:

4. Close the outlet valve and open the inlet valve to apply 80 PSI or 80mV to both PT1 and PT2. Adjust the PT1 gain until dP reads zero mV.

5. Close the inlet valve and open the outlet valve. PT1 and PT2 should both be at zero PSI and zero mV. Adjust the dP zero until dP reads zero mV.

6. Close the outlet valve and open the inlet valve. Check to see if dP still reads zero. If not then adjust PT1 gain until it does equal zero. Repeat steps (4) and (5) until dP equals zero when PT2 = 0 mV and PT2 = 80 mV.

11.5 Calibration of dP

Lower the pressure in the captive air tank until PT2 reads about 15 mV (= 15 PSI). Make sure the **Red** range is selected and repeat the following steps:

7. Close the outlet valve and open the inlet valve. Make sure dP still reads zero. If not, readjust the dP zero pot. Write down the value of PT2.

8. Open the outlet valve while the inlet valve is still open. There will now be a pressure drop across the **Red** tube equal to the value of PT2 you wrote down in step (7). If dP does not equal PT2 in step (7), then adjust the dP gain pot until it does.

9. Close the outlet valve while the inlet valve is still open. See if dP still reads zero. If not, then adjust the dP zero pot until it does. Then repeat step (7) and (8). The objective is to get dP to read zero when there is no flow across the **Red** tube and to make dP = PT2 (from step (7)) when there is flow across the **Red** tube.

You are now finished with the electronic calibration of the A/D board. You may also want to calibrate PT2 output in mV versus a test pressure gage because the calibration table needs the conversion between mV and MPa. If you do not have a test pressure gage more accurate than 1%, then use the nominal factory conversion factor of 6.8966E-3 MPa/mV, which applies to all PX26 transducers with 1% accuracy.

11.6 Setup of communications with your balance

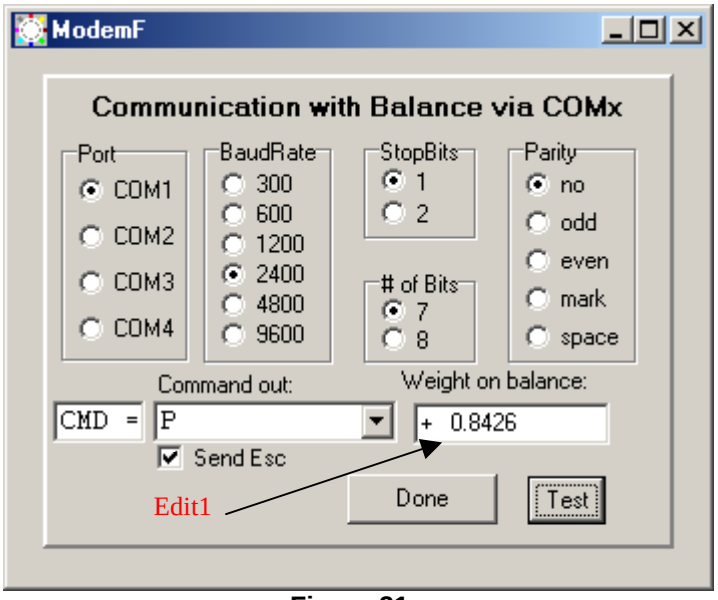


Figure 21
Communication with the balance

In order to set up communication with your balance, you should connect the balance to the computer via a known COM port. Turn on the balance and click on the **"COMx Setup"** button.

The window in figure 20 should be displayed. This window is set up to communicate with a Mettler balance connected to COM2 set for 2,400 baud, 1 stop bit, 8 bits, no parity. The command string to read a weight is 'SI'. Another common command string is 'P' either with or without ESC. If ESC is needed, be sure the **"Send Esc"** box is ticked. You can test the communication by clicking on the **"Test"** button. If it works, the **"Edit1"** text box should display current weight shown on the balance. If the default settings do not work for you then you must consult your balance manual for the communication parameters and you must edit your own **"Command out"** text by clicking on the text box with SI and then changing it to the command expected by your balance. You may also need to consult your computer manual to figure out what COM ports are available on your computer and which one you are currently using!

NOTE

Some balances require custom cables and personal experience is that some balance manuals have INCORRECT information about cable requirements so GOOD LUCK!

11.7 Calibration of a Flow Range

When the calibration window appears, the table is filled with the most recently collected set of calibration data. (If the table is blank then that is okay.) In Figure 21 (below) the **Red** range has been selected by clicking on the radio button. This causes the values in the table to be displayed in the graph to the left.

In order to append new values to the current table, all you need to do is click on the **"Start"** button. This will cause the software to read dP every 2 seconds and the balance every 4 seconds. When the cumulative weight change exceeds 0.02 g, a new set of values for Flow and dP will appear in the memo boxes to the left of the **"Start"** button. For the lower ranges (**Green** and **Orange**) a value of 0.02 is okay, but for higher flow rates you will want to select a bigger dW by clicking on the drop-down text box and selecting a higher value. You can also

select to have values updated after a fixed dT by clicking on the **"On Fixed dT"** option. The default time interval is 32 s, but you can select other values by clicking on the drop-down text box. You should allow data to accumulate until you get two values of flow that agree within 1% (= stable value). When you have a stable value, you can enter the value in the table by clicking on the **"Select Last F,dP"** button. The value will be added to the first blank space on the table.

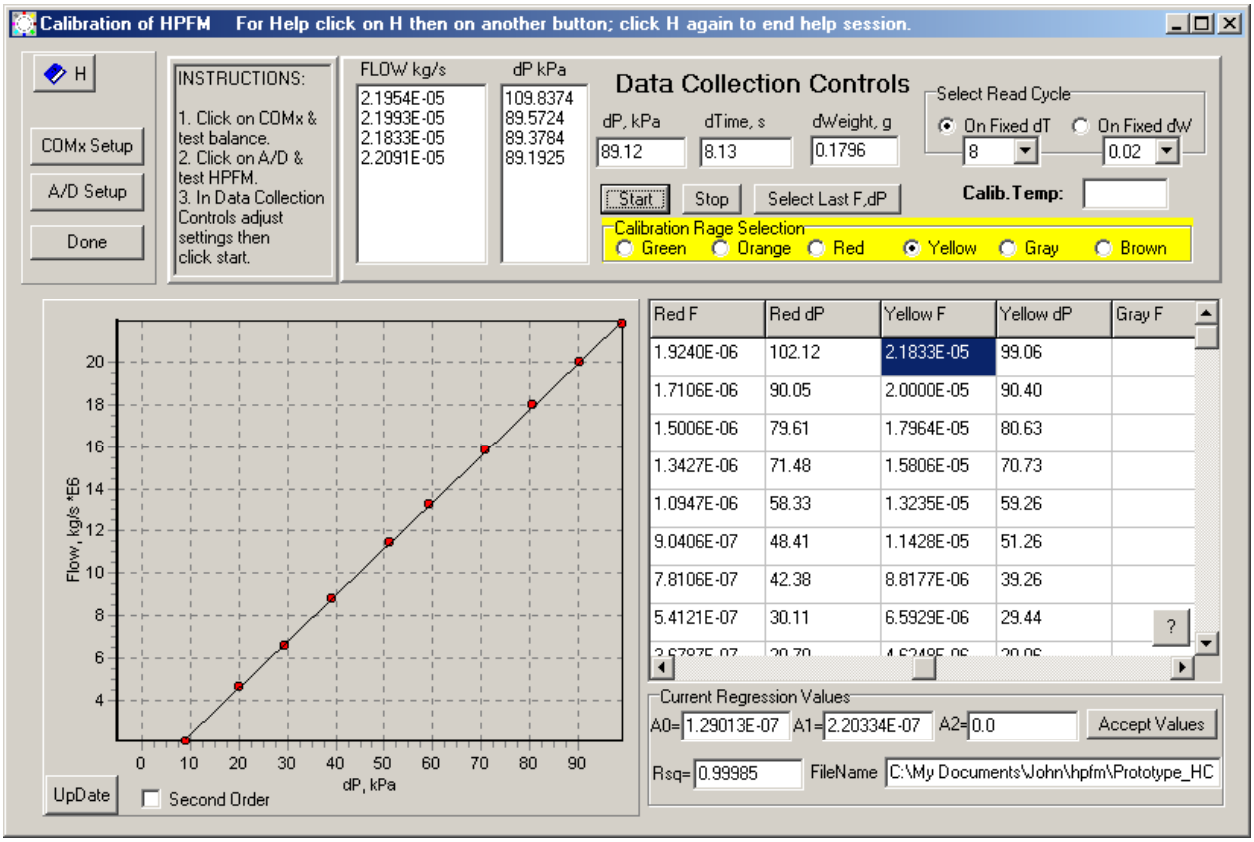


Figure 22
Calibration of a Flow Range

NOTE: if you need to collect a totally new table of values for a specific range you have to manually delete all the unwanted values. Clicking on each data value to be deleted until it is highlighted in blue, and then press the **"Del"** key on your keyboard does this. You need to delete only the flow values; the dP values will be overwritten.

It is best to measure at least 10 values of flow versus dP for dP values from 0 to 120 kPa (0 to 17 PSI). To view the regression click on the **"Update"** button in the lower left corner of the graph. For the higher flow ranges (**Gray** and **Brown**) you will also need to click on the **"Second Order"** tick box to get a second order polynomial regression. Each time you click the **"UpDate"** button, the current values of the regression coefficients will also be updated in the lower right side of the window. When you have finished the regression for the **Red** range (or whatever range you are on) you can save the regression values by clicking on the **"Accept Values"** option. You will be asked for a filename for the new regression eg HPFM_ABC.cal Repeat this process for the remaining five ranges and use the same file name for all six ranges.

NOTE: It is best to do a calibration in a room with stable temperature (± 0.5 °C) because the calibration factors change 2.3% per degree C. If the room temperature changes more than 1 °C, then keep a record of room temperature for each calibration range and enter the mean value in the **"Calib. Temp."** text box. This value is also saved in the calibration table**.**

CAUTION! KNOWN BUG

The calibration procedure SOMETIMES overwrites a vital value that specifies the conversion between PT2 in mV to MPa. The conversion factor should be 6.8966E-03. This does not happen every time so the cause of the bug has not yet been located. A much smaller value, which makes the HPFM report very small or no pressure increase during pressurization has overwritten the value. The solution to the bug is to manually enter the correct calibration factor as explained below.

11.8 Setting the Calibration Factors

The '**View/Set Calib**' allows you to view the numerical values of the calibration factors and to manually edit and save them using the window that comes up as illustrated in figure 22 below:

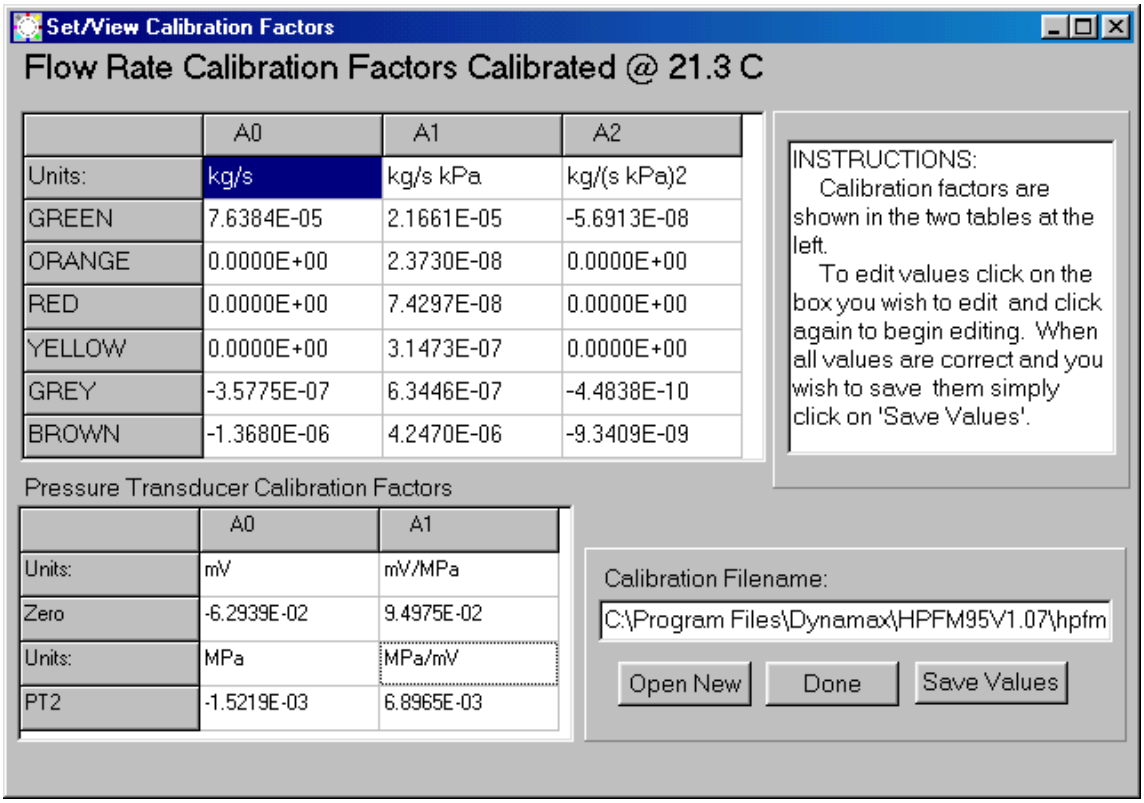

Set/View Calibration Factors
Flow Rate Calibration Factors Calibrated @ 21.3 C
A0 A1 A2
Units: kg/s kg/s kPa kg/(s kPa)2
GREEN 7.6384E-05 2.1661E-05 -5.6913E-08
ORANGE 0.0000E+00 2.3730E-08 0.0000E+00
RED 0.0000E+00 7.4297E-08 0.0000E+00
YELLOW 0.0000E+00 3.1473E-07 0.0000E+00
GREY -3.5775E-07 6.3446E-07 -4.4838E-10
BROWN -1.3680E-06 4.2470E-06 -9.3409E-09
INSTRUCTIONS:
Calibration factors are shown in the two tables at the left.
To edit values click on the box you wish to edit and click again to begin editing. When all values are correct and you wish to save them simply click on 'Save Values'.
Pressure Transducer Calibration Factors
A0 A1
Units: mV mV/MPa
Zero -6.2939E-02 9.4975E-02
Units: MPa MPa/mV
PT2 -1.5219E-03 6.8965E-03
Calibration Filename:
C:\Program Files\Dynamax\HPFM95V1.07\hpfm
Open New
Done
Save Values

Figure 23
View/Set Calib window

11.9 New Calibration File

From the **"File"** menu select the **"NewCalFile"** option. This brings up a load-file dialog box and permits you to select a new calibration file (if alternate files exist on disk). All calibration files carry the extension '.CAL'

You should reset calibration factors **ONLY** if you have new and **reliable** calibration data. The data are stored in a file named: HPFM.CAL. It is a good idea to make a copy of the old file with a new name if you plan to change the values or keep a backup copy in case you make a mistake.

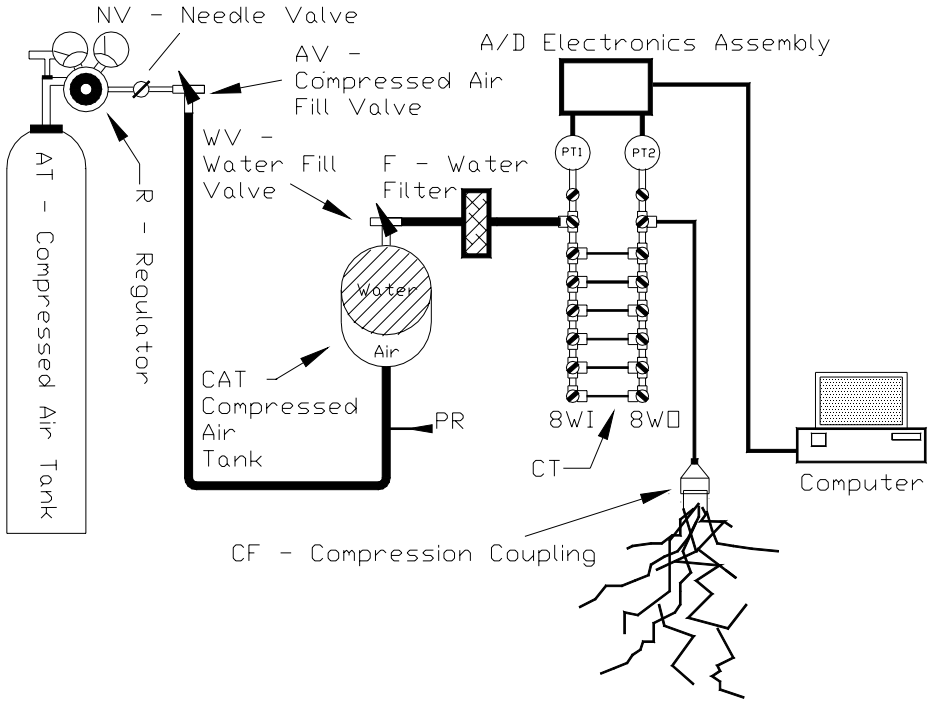
11.10 Suggested Spare Parts

1. "O" Rings, Catalog No. 1401, set of 10
2. "O" Rings, Catalog No. 1402, set of 10
3. Rubber Compression seals, No. 6.5, Set of 6
4. Rubber Compression seals, No. 10, Set of 9
5. Rubber Compression seals, No. 13, Set of 9
6. Tubing, Catalog No. 1535, .005" I.D. HPLC (Red), 5'
7. Tubing, Catalog No. 6406-62, Rigid Teflon C-9, 5'
8. Assorted compression fittings, Catalog No. 1310

12. APPENDIX I - Parts List

No.	Amount	Part No.	Description
1	1 Unit	HPFM	Standard HPFM Unit
2	1 pack	1401	Small o-rings (10 per)
3	1 pack	1402	Large o-rings (10 per)
4	2	1310	Plastic Compression Caps
5	5 FT	1535	(.005") I.D. x 1/16" O.D. “Red” Bleed Tubing
6	5 FT	1540	(.042 ") 1mm I.D. HPLC “Natural” Connection Tubing
7	6 FT	6406-62	Teflon FEP C-9 rigid “Clear” Connection Tubing
8	1 EA	HPCC1-4 1001	1-4mm Two way connector
9	1 EA	HPCC4-10 1008	4mm-10mm universal connector
10	2 EA	RSW502	Cone washer 3/8" for 8-10 mm range
11	1	HPCC10-20	10-20mm Compression Coupling
12	1	HPCC19-36	19-35mm Compression Coupling
13	1	HPCC-36-55	34-50mm Compression Coupling
14	1	Set	10-20mm Al. Compression Rings (2)
15	1	Set	19-35mm Al. Compression Rings (3)
16	1	Set	34-50mm Al. Compression Rings (3)
17	6	#6 1/2	Coupling Seals (drilled)
18	9	#10	Coupling Seals (drilled)
19	9	#13	Coupling Seals (drilled)
20	1	G4	Silicone Coupling assembly Grease
21	1		12V 4AH Battery Charged and tested
22	1		**Battery Charger 12V DC out (Center POS) 100/240 VAC IN**
23	6 FT		Serial /Parallel 6 Ft Cable (Male - Female)
24	1		Spare 9V Alkaline Battery
25	1		2000 PSI regulator (Only for HPFM-C)
26	1	B-4-HN	Hex Nipple Installed on Regulator (Only for HPFM-C)
27	1		Nitrogen Tank (Only for HPFM-C)
28	1 EA	MP2176	Air hose 5' with Male Couple, Quick Disc
29	2		Wheels & 1.75 Bolts (Not on HPFM-L)
30	1		Pack Frame (Not on HPFM-L)
31	1		Pack Straps, Back Pad (Not on HPFM-L)
32	1	101P	Custom Refill Kit & Quick Disconnect
33	1		3cc & 30cc syringes w/ 2 blunt needles & 1 Razor blade
34	2 oz.		Algaecide
35	1		Manual
36	1 Set		Calibration Curve, CD and Hardcopy
37	1		XP compatible HPFM software
38	1		Packaging Carton w/ Padding

13. APPENDIX II



Bill of Materials

AV = Compressed Air Valve
WV = Water Valve
NV = Needle Valve
F = Water Filter
PC = Purge Cap on Water Filter (F)
CAT = Captive Air Tank
SB = supply box for storage of small parts and location of 12 VDC 4.5 Amp Hour Battery.
A/D = location of A/D circuit in the supply box.
PS = power switch to the A/D circuit.
OQ = orange quick fit Swagelok connector for adding water to the CAT.
GQ = red quick fit Swagelok connector for connection to Pressure Regulator.
PT1 = Pressure Transducer #1 on inlet side
PT2 = Pressure Transducer #2 on outlet side
8WI = 8-way manifold on inlet side
8WO = 8-way manifold on outlet side
IV = Flow Inlet Valve on inlet 8-way valve.
OV = Flow Outlet Valve on outlet 8-way valve.
DP = Digital Pressure Gage
SRV = Safety Release Valve that prevents the CAT from being pressurized more than 90 PSI.

14. APPENDIX III - Reference List

43. Tyree, M.T., Patino, S.,Benink, J., Alexander, J., (1994). *Dynamic measurements of root hydraulic conductance using a high-pressure flowmeter in the laboratory and field*. Journal of Experimental Botany, Vol. 46, No. 282, pp. 83 - 94.
A new high-pressure flowmeter (HPFM) is described that is capable of rapid water-flow measurements. This article describes the HPFM, presents the theory of dynamic flow measurements, discusses sources of error, presents evidence that dynamic measurements of K_r in *Ficus maclellandi* (and six other tropical species from Panama) yield the correct result, and demonstrates the use of the method under field conditions in Panama on *Cecropia obtusifolia* and *Palicourea guianensis.*

51. Zotz, G., Tyree, M.T., Carlton, M.R., (1998). *Hydraulic architecture and water use of selected from a lower montane forest in Panama*., Trees, No. 477, pp. 1 - 8.
Plant water relations of nine woody species were studied in a lower montane rain forest in Panama. This data provides a partial test of the hypothesis that hydraulic architecture of lower montane species might limit transpiration and thus leaf size or nutrient transport (as suggested by J. Cavelier and E.G. Leigh, respectively). Another objective of this study was a comparison of two different methods to measure hydraulic conductance and leaf specific conductance of stem segments.

52. Tyree, M.T., 1997. *The Cohesion-Tension theory of sap ascent: current controversies.,* Journal of Experimental Botany, Vol. 48, No. 315, pp. 1753 - 1765.
In recent years, the Cohesion-Tension theory of sap ascent in plants has come under question because of work published by Professor Ulrich Zimmermann and colleagues at the University of Würzburg, Germany. The purpose of this review is to state the essential and testable elements of the C-T theory, summarize the negative evidence for the C-T theory, and review critically the positive evidence for the C-T theory and the evidence that the Scholander-Hammel pressure bomb measures xylem pressure potential (P_X) correctly, because much of the evidence for the C-T theory depends on pressure bomb data.

55. Tsuda, Makoto and Tyree, M.T., (1997). *Whole-plant hydraulic resistance and vulnerability segmentation in Acer saccharinum.,* Tree Physiology Vol. 17, pp. 351 - 357.
Hydraulic properties were studied in *Acer sacharinum L.*, a riparian species that also grows well on a dry soil when transplanted. Hydraulic resistances were measured by two independent techniques: a new high-pressure flowmeter (HPFM) method and a conventional evaporative flux (EF) method. Vulnerability to cavitation was also investigated on petioles, stems and roots using hydraulic conductivity technique.

70. Tyree, M.T., Velez, Virginia, Dalling, J.W., (1998). *Growth dynamics of roots and shoot hydraulic conductance in seedlings of five neotropical tree species: scaling to show possible adaptation to differing light regimes.* Oecolgia Vol. 114, pp. 293 - 298.
The dynamics of growth (shoot and root dry weights, surface areas, hydraulic conductances, and root length) were measured in seedlings of five neotropical tree species aged 4-16 months. The species studied included two light-demanding pioneers and three shade-tolerant young or old forest species. Growth analysis revealed that shoot and root dry weights, hydraulic conductances and leaf area all increased exponentially with time. The advantages of scaling hydraulic parameters to leaf surface area are discussed in terms of the Ohm's law analogue of water flow in plants.

71. Tyree, M.T., Sobrado, M.A., Stratton, L.J. (1998). *Diversity of Hydraulic conductance in leaves of temperate and tropical species: Possible causes and consequences.* Journal of Tropical Forest Science.
Liquid-flow-pathway hydraulic conductances (Kl) were measured in leaves of 24 species of temperate and tropical plants. Whole shoot conductances were measured by transient (Kl) and quasi-steady state(Kq) methods.Implications of low leaf conductances on the measurement of xylem pressure potential with a Scholander-Hammel pressure bomb are also discussed.

79. Cochard, H., Martin, R., Gross, P., Bogeat-Triboulot, M.B. (2000). *Temperature effects on hydraulic conductance and water relations of Quercus robur L.* Journal of Experimental Botany, Vol. 51, No. 348, pp. 1255 - 1259.
The effects of temperature on root and shoot hydraulic conductances were investigated for *Quercus robur* L. saplings. In the first experiment, conductances were measured with the HPFM on excised shoots and detopped root systems. In the second experiment, the impact of temperature-induced changes in g_{root} on sapling transpiration (E) and leaf water potential was assessed. Intact plants were placed in a growth cabinet with constant air and variable soil temperatures. The results illustrate the significance of g_{plant} for the stomatal control of transpiration and the significance of temperature for tree water transport.

15. APPENDIX IV – Glossary

A/D
The output of the pressure transducers is logged using a custom-designed, dual channel A/D circuit with 12 bits plus sign accuracy. The location of A/D circuit in the supply box. The HPFM is supplied with Windows software for controlling the A/D circuits, logging data, and for preliminary data analysis.

Air Valve, AV

Capillary Tubes, CT
The capillary tubes are 1.5 mm OD HPLC tubing 0.16 to 1.5 m in length.

Captive Air Tank, CAT
The Captive Air Tank (CAT) is a two-compartment tank. The upper compartment contains water and the lower section can be pressurized with air. A flexible rubber diaphragm separates the two compartments. The large stainless steel CAT allows you to pressurize the water without the air and water mixing together. The CAT will hold 7.0 liters of water and it is best to refill while there is still at least 0.5 liters of water in the tank. The Captive Air Tank (CAT) should be filled only with 'degassed water', i.e., water with air dissolved at less than saturating concentrations of O_2 and N_2 at atmospheric pressure. It is important to prevent air bubbles from entering the Captive Air Tank (CAT) because these bubbles will re-saturate the degassed water.

Cubic Feet, Cu. Ft.
The compressed air tank for the HPFM has a volume of 24 cubic feet.

Differential pressure, dP
Water flow across the selected CT causes a differential pressure (dP) drop ($dP = P_1 - P_2$) measured with PT1 and PT2, respectively. If the software recognizes that the differential pressure between PT1 and PT2 is over 18 milli-volts or 18 PSI (124 kPa), then it will give you a message telling you that the HPFM is out of range. The working range for the differential pressure is 0 to 18 milli-volts. The desired range is 8 to 15 milli-volts. When you are working with root systems, the differential pressure will normally increase the longer you have the HPFM coupled to the root.

DT

DW

Water Filter, F

Flow to compress air bubbles, F_b

Elastic flow, Flow associated with elastic swelling, F_e
In order to reduce elastic flow, F_e, always use small-diameter, rigid outlet tubing when using the two lowest flow rate ranges (Green and Orange).

Rate of passage through the roots, F_h
If the flow through the object measured is a linear function of applied pressure difference between the outlet of the HPFM and atmospheric pressure.

GQ
Green quick fit connector for adding air to CAT.

Hydraulic Conductance
It is an apparatus designed to perfuse water into an object while rapidly changing the delivery pressure and simultaneously measuring flow. The slope of flow plotted versus pressure equals the hydraulic conductance of the object. While measurement of the Transient is simply graphing a line of increasing pressure and flow, Regression is the "best fit" of that graphical line and calculating the slope. That slope is the hydraulic conductance of the measured plant.

Hypodermic syringe
Air must be removed by disconnecting PT2 from the 8WO and injecting water using a hypodermic needle and syringe. The HPFM comes with a hypodermic syringe that has a blunt point "needle".

Inside Diameter, ID
The inside diameter that is 9 mm of a nylon-reinforced Tygon tubing where pressurized water flows from the CAT to the 8-Way Inlet manifold (8WI).

Flow Inlet Valve, IV
on inlet 8-way valve.

K
uncorrected conductance

Kpa
Measurement for pressure in Kilo-Pascal's

K root conductance, K_r

K_{CT}
Conductance of the capillary tubes.

LPT1, LPT2, or LPT3
A connection used to connect the HPFM to a PC. When the HPFM program starts, the program automatically tests for basic communication with the HPFM by testing to see if the HPFM is connected to the computer through one of three possible printer port connections.

Mpa
Measurement for pressure

Mohms

MV
Measurement in milli-volts

n
the number of moles of gas in the bubble

Needle Valve, NV
Used to regulate the rate of increasing pressure. The rapid change in water pressure is achieved using the needle valve. Through the needle valve, the pressure regulator keeps the compressed air at a steady pressure of 4 to 5 MPa (580-725 PSI).

Outside Diameter, OD

OQ
Orange quick fit connector for adding water to CAT.

OV
Flow Outlet Valve on outlet 8-way valve.

P_i
Initial gas pressure.

PSI
Measurement of pressure in pounds per square inch.

P_2
The outlet pressure relative to atmospheric pressure applied pressure.

Purge Cap, PC
Air is purged using the red purging caps located on the top and the bottom of the filter near the outer edge of the round body of the filter.

PR
Pressure Regulator that prevents accidental over-pressurization.

Pressure transducers, PT1
Pressure Transducer #1 on inlet side.

Pressure transducers, PT2
Pressure Transducer #2 on outlet side.

Power switch, PS
to the A/D circuit.

Purge Valve, PV

Pressure Regulator, R
Delivers compressed air from the compressed air tank (AT).

Quasi-steady State Measurements of Hydraulic Resistance, R_{QS}
During steady-state measurements of R_{QS}, water flow and applied pressure are both constant and by definition the water flow into the object measured equals the flow out of the object.

Unknown hydraulic resistance, R_U
The unknown hydraulic resistance of a quasi-steady state measurement.

Supply Box, SB
For storage of small parts and location of 12 VDC 4.5 Amp Hour Battery.

Safety Release Valve, SRV
Prevents the CAT from being pressurized more than 90 PSI.

T
The Kelvin temperature at which K was measured.

μm

Initial volume, V_i

viton "O" rings
When removing the HPLC compression-fitting cap (the colored tubing fitting at the top of the compression coupling), there should be at least 2 small viton "O" rings inside the connection.

Volume of the system, V

V_o
The initial volume of the system at P2 = 0

V_b
The volume of a bubble at absolute gas pressure P_b

Water Valve, WV
Allows water flow into the filter and subsequently into the valve manifold (8WI and 8WO) system at P2 = 0.

8WI
An Omnifit, 8-Way, HPLC valve of octagonal geometry with 8-tubes emerging from a common point in the center and each tube terminated by a valve.

8WO
An 8-way manifold on outlet side